Neurology

OFFICIAL JOURNAL OF
THE AMERICAN ACADEMY OF NEUROLOGY

NOVEMBER--1961

Volume 11 - Number 11

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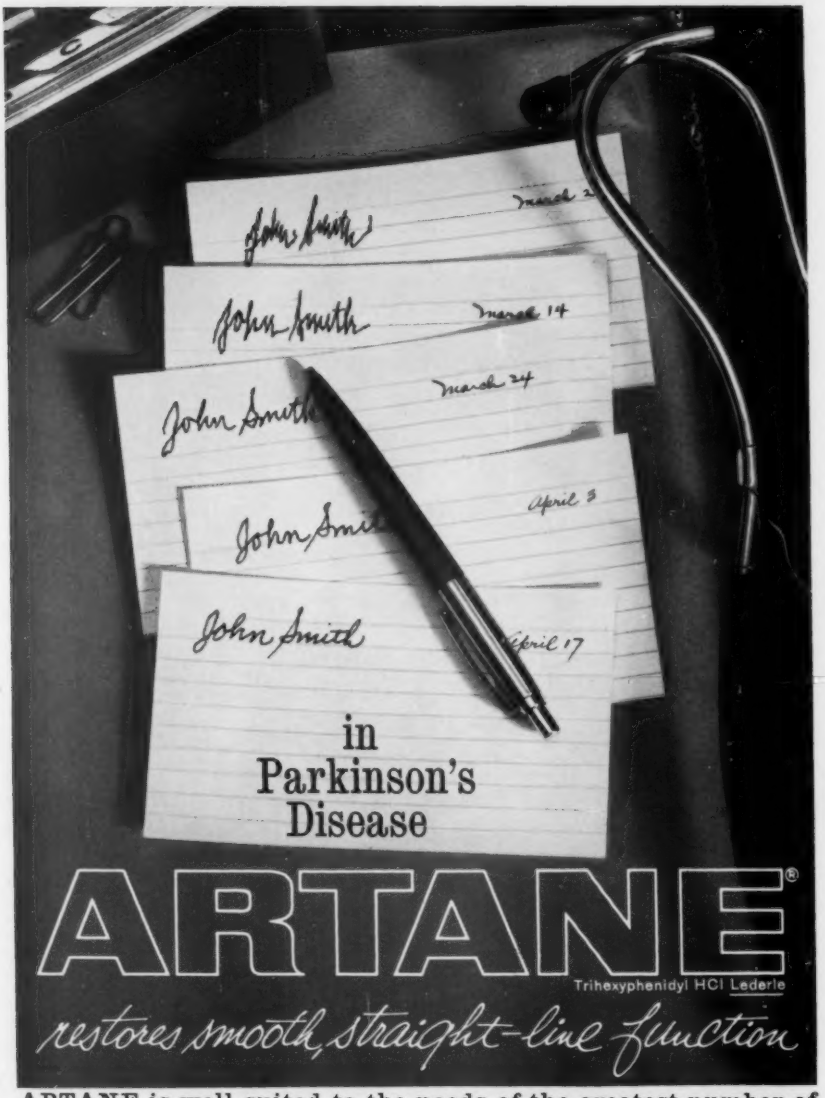

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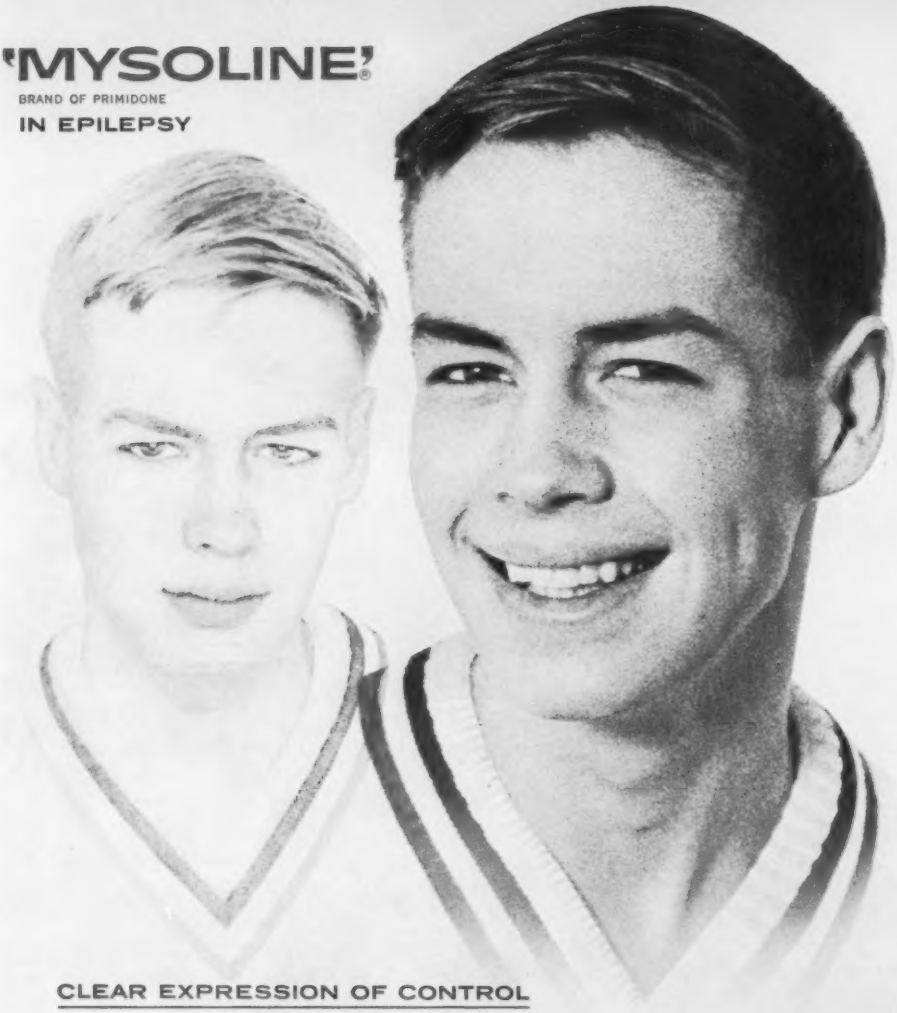

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Volume 11 **Neurology** *Number 11*

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NOVEMBER 1961 • *Contents*

Muscle Blood Flow Studies in Muscle-Contraction Headaches 935
YUSUF ONEL, M.D., ARNOLD P. FRIEDMAN, M.D., and JACOB GROSSMAN, M.D.

Long-Latency Cerebellar Responses in Cerebellar Pedunculi and Cortex 940
SHIGEYUKI DEURA, M.D.

Cardiorespiratory Syndrome of Extreme Obesity with Papilledema: Report of a Fatal Case with Electroencephalographic, Metabolic, and Necropsy Studies 950
J. S. MEYER, M.D., J. GOTHAM, M.D., Y. TAZAKI, M.D., and F. GOTOH, M.D.

Fibrinolysis and the Nervous System 959
STUART A. SCHNECK, M.D., and KURT N. von KAULLA, M.D.

Morphologic Findings in Studies of Regeneration of Synapses in the Mammalian Spinal Cord 970
C. C. TURBES, D.V.M., and L. W. FREEMAN, M.D.

Autotransplantation of Anaplastic Astrocytoma into the Subcutaneous Tissue of Man 977
ARTHUR F. BATTISTA, M.D., WILLIAM BLOOM, M.D., MAURICE LOFFMAN, M.D., and IRWIN FEIGIN, M.D.

Effect of Trypan Red on Columbia-SK Virus Pathogenicity 982
BENJAMIN V. SIEGEL, Ph.D., ROBERT B. AIRD, M.D., and WILLIAM W. ANDERSON, M.D.

Immunoelectrophoretic Studies of Cerebrospinal Fluid: Investigations in Patients with Tumor in the Central Nervous System 989
E. SVENNILSON, M.D., S. J. DENCKER, M.D., and B. SWAHN, M.D.

(Continued on following page)

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Contents—*Continued*

Chronic Cerebrospinal Fluid Otorrhea: Report of a Case Associated with Chronic Infection of the Ear 996
JOHN MEALEY, JR., M.D.

Hereditary Interstitial Nephritis Associated with Polyneuropathy 999
OSCAR S. M. MARIN, M.D., and H. RICHARD TYLER, M.D.

Ability To Distinguish Two Cutaneous Stimuli Separated by a Brief Time Interval 1006
JOSEPH B. GREEN, M.D., CHARLES L. REESE, M.D., J. J. PEGUES, M.D., and FRANK A. ELLIOTT, M.D.

Effect of Serotonin on Behavior, Electrical Activity of the Brain, and Seizure Threshold of the Newly Hatched Chick: With a Note on the Production of Seizures Presenting 14-per-second and 6-per-second Positive Spikes 1011
K. N. HEHMAN, B.S., A. R. VONDERAHE, M.D., and J. J. PETERS, Ph.D.

Delayed Focal Convulsive Seizures after Head Injury in Infants and Children: A Syndrome That May Mimic Extradural Hematoma 1017
KENNETH E. LIVINGSTON, M.D., and MOHSEN MAHLOUDJI, M.B., CH.B.

Case Report: Fatal Electrolyte Imbalance Secondary to Cerebrospinal Fluid Fistula in an Infant 1021
VINCENZO V. LOMBARDI, M.D., and HAROLD C. VORIS, M.D.

Book Reviews 1023

INFORMATION FOR *Authors*

NEUROLOGY, the official publication of the American Academy of Neurology, has been instituted to provide a medium for prompt publication of articles dealing with neurology and its cognate fields and to stimulate individual investigation in these various fields.

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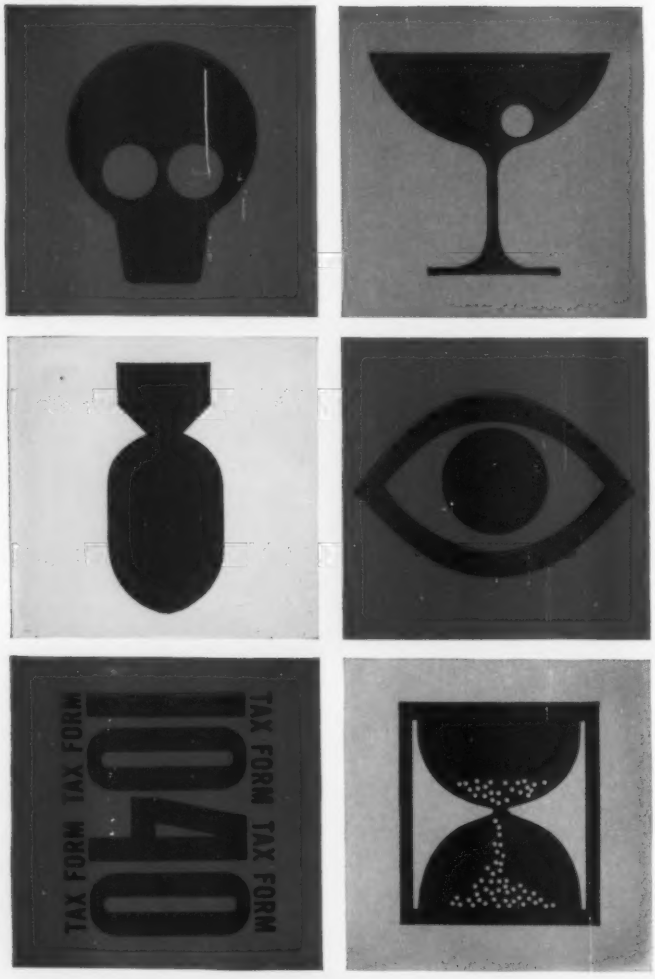

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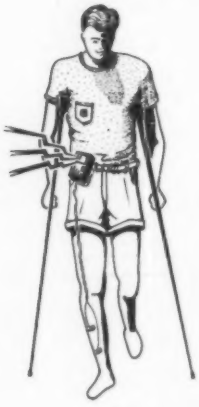

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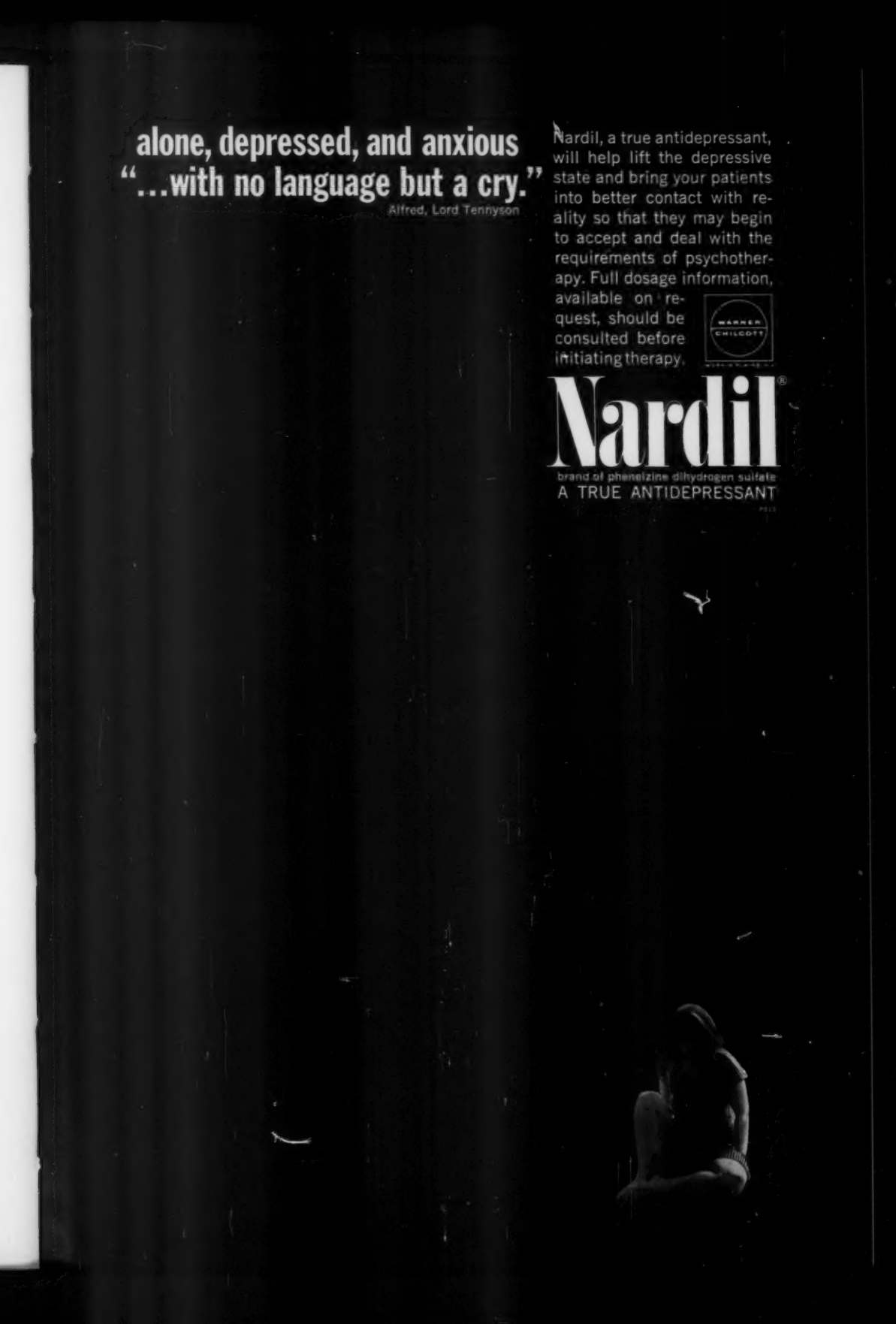

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Neurology Volume 11 • Number 11

Muscle blood flow studies in muscle-contraction headaches

Yusuf Onel, M.D., Arnold P. Friedman, M.D., and Jacob Grossman, M.D.

IT HAS BEEN POSTULATED that pain of muscle-contraction headache occurs partly as a result of sustained contraction of skeletal muscles about the head and partly as a result of local tissue ischemia in the affected area.

Evidence for the presence of ischemia is highly indirect, based on the observed vasoconstriction in the bulbar conjunctiva[1] and markedly diminished pressure pulse waves of the local cranial arteries during the headache.[2–4] Moreover, administration of ergotamine tartrate, a vasoconstrictor, fails to relieve and often aggravates the headache; secondly, ipsilateral carotid artery compression may augment the intensity of head pain, while massage, warmth, or ingestion of moderate amounts of alcohol may relieve this kind of headache.

However, a more direct approach to the problem of ischemia requires measurement of blood flow in the involved muscles.

Kety,[5,6] Wisham, Yalow, and Freund,[7,8] Smith and Quimby,[9] Franke and associates,[10] and Semple, McDonald, and Ekins[11] have demonstrated that the local tissue clearance of radioactive sodium (Na^{24}) may serve as an index of effective blood flow. (Effective blood flow refers to that flow which traverses capillaries and therefore is involved in the exchange of nutriment and metabolites.) If Na^{24} is introduced into the tissue in question, its clearance or removal rate from the tissue depends upon, and therefore reflects, local capillary circulation.

This study was undertaken to determine the effective blood flow in the neck muscles in muscle-contraction headache. Muscle-tension headaches[12] may be defined as periodically recurring headaches of varying frequency and severity, usually persisting for several hours or even days. They occur without prodromas and are commonly bilateral. The pain usually is described as an ache or sensation of pressure and is frequently occipital and suboccipital, al-

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Presented at the thirteenth annual meeting of the American Academy of Neurology, Detroit, April 29, 1961.

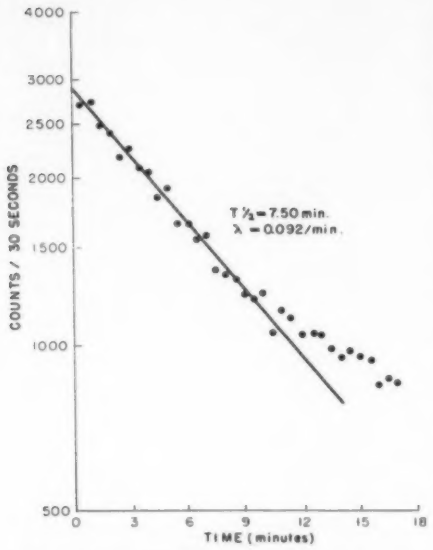

Fig. 1. Disappearance of Na^{24} from site of injection showing half life ($T\frac{1}{2}$) and fractional rate of removal (λ)

though it may be frontal or sensed as a band around the head. In patients of this type, muscle contraction is part of excessive bodily reactions to life stress.

METHODS

The subjects of these measurements were patients attending the Headache Unit at Montefiore Hospital. On the basis of history, physical and neurologic examinations, and ancillary tests including skull and chest roentgenograms, electroencephalography, and perimetry, they were found to be in good health apart from their headaches. All were requested not to eat or drink during the three hours preceding the tests, which were done during the late afternoon. Room temperature was kept comfortable.

In each case, the subject was kept at rest in the prone position for ten to fifteen minutes before testing. After the intradermal introduction of sufficient Metycaine (1%) to produce a small bleb and anesthesia, 0.2 cc. (containing 4 μc.) of isotonic $Na^{24}Cl$ solution was injected via a 1½-in. No. 22 needle into the splenius capitis muscle. A Geiger-Müller body counter was then placed over the site of injection and, after five minutes were allowed for local equilibration, counting was carried out by recording the total counts every thirty seconds for twenty to twenty-five minutes.

After correction for background activity, the data were plotted semilogarithmically as a function of time. Clearance or fractional removal is expressed as the slope of the straight line obtained (Fig. 1). The half life is the time required for the activity at any time to fall to one-half its previous value. The fractional rate of removal of isotope (λ) is equal to the natural logarithm of 2 (0.693) divided by the half life: $\lambda = \frac{0.693}{T\frac{1}{2}}$.

RESULTS

A total of 71 studies were performed on 56 patients, of whom 5 were nonheadache control volunteers; 32 studies were carried out in males and 39 in females. Ages ranged from 18 to 65. The right splenius capitis was injected twenty-four times, the left forty-seven. Age, sex, and side of injection did not influence the Na^{24} clearance rates (Figs. 2 through 5).

As shown in Figure 5, 50 studies were performed on headache patients in the absence of significant headache, while 16 were carried out during severe headache. In the absence of headache, in 50 studies on 44 patients, the slopes (fractional removal rates) varied from 0.05 to 0.14 per minute (mean: 0.094 ± 0.025 per minute). During headache (16 patients), the slopes ranged from 0.09 to 0.17

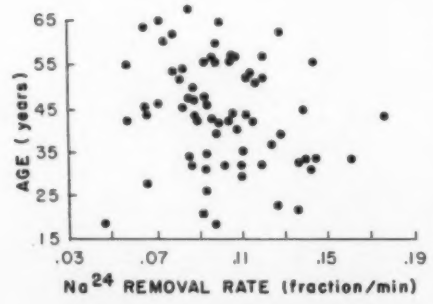

Fig. 2. Lack of correlation between age of subject and tissue clearance of Na^{24}

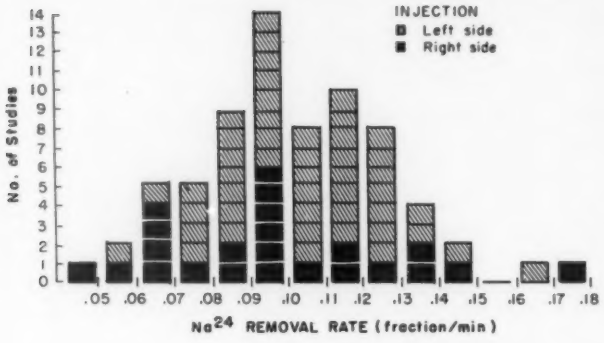
INJECTION
Left side
Right side
No. of Studies
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
.05 .06 .07 .08 .09 .10 .11 .12 .13 .14 .15 .16 .17 .18
Na24 REMOVAL RATE (fraction/min)

Fig. 3. Clearance rates on injections in left and right sides. See Figure 4.

per minute (mean: 0.12 ± 0.021 per minute), a statistically significant difference ($P<0.01$).

Comparison between studies performed during headache and those in either patients without headache or control subjects revealed significant differences ($P = 0.01$ to 0.001) (Fig. 6). However, comparison between patients in the absence of headache and the control subjects failed to elicit a significant difference, suggesting that the increased clearance of Na^{24} is associated with the presence of the headache at the time of measurement rather than its history in a given patient. To support further this observation, each of 5 patients were studied on 2 separate occasions—during and in the absence of headache. As can be seen in Figure

Slopes ($min.^{-1}$)

Patient	Right Side	Left Side	Difference
Q	0.083	0.087	-0.004
R	0.099	0.084	+0.015
S	0.090	0.099	-0.009
T	0.086	0.106	-0.020
U	0.083	0.064	+0.019
V	0.098	0.090	+0.008
X	0.131	0.136	-0.005
Mean:	0.096	0.095	0.001

P=0.6

Fig. 4. Clearance rates of Na^{24} on both sides in each of 7 subjects

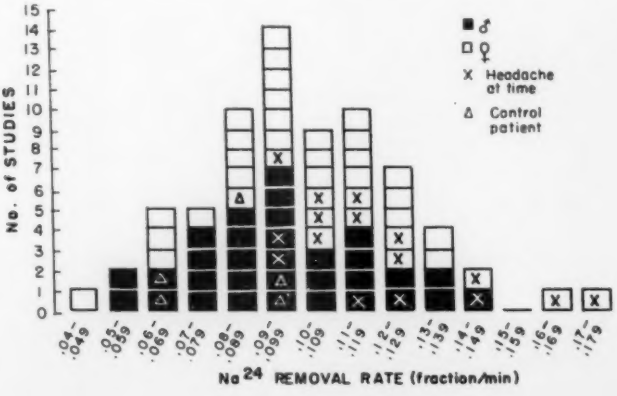

Fig. 5. Summary of tissue clearances of Na^{24} in 71 studies in 3 population groups. Note more rapid fractional removal during headache.

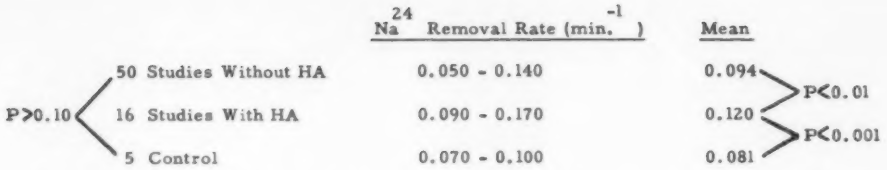

Fig. 6. Comparison of Na^{24} removal rates in patients during and in absence of headache and in control subjects without history of headache

7, in every case, the clearance of Na^{24} was significantly greater ($P<0.01$) during headache (without headache: mean = 0.087; during headache: mean = 0.136). Conversely, 6 patients studied repeatedly in the absence of headache failed to exhibit significant differences between the 2 studies (Fig. 7).

DISCUSSION

The results indicate that, during the type of headache described, the effective (capillary) blood flow in the muscle studied or permeability to Na^{24} is increased. Of the two factors, blood flow probably is more important.

The likelihood of muscle ischemia is there-

Slopes ($min.^{-1}$)

Patient	Without HA	With HA	Difference
A	0.087	0.141	+0.054
B	0.097	0.141	+0.044
C	0.089	0.112	+0.023
D	0.065	0.112	+0.047
E	0.098	0.173	+0.075
Mean:	0.087	0.136	+0.049

$P<0.01$

Patient	Without HA	Without HA	Difference
K	0.115	0.083	-0.032
L	0.066	0.071	+0.005
M	0.100	0.084	-0.016
N	0.078	0.103	+0.025
O	0.112	0.128	+0.016
P	0.110	0.107	-0.003
Mean:	0.097	0.096	-0.005

$P = 0.6$

Fig. 7. Influence of headache at time of study

fore reduced since muscle contraction headache appears to be associated with increased blood flow. However, in the presence of sustained muscle contraction, even such increased blood flow does not exclude the possibility of relative ischemia, that is, insufficient blood flow for the metabolic needs of the active muscle. It would be of interest to investigate blood flow in other muscles during tension headaches.

The existence of increased flow does not per se imply a causative role. Conceivably, it may be an effect or concomitant of the factor producing the headaches. Nevertheless, the finding of altered flow suggests areas of therapeutic approach. Moreover, these data provide a basis for extending such tissue clearance measurements to other types of headache.

Acknowledgment is made to Dr. L. H. Wisham for his advice and suggestions.

Muscle-contraction headache is part of the individual reaction to life stress referred to by the ambiguous term "tension headache." The term "muscle-contraction headache" was suggested by the panel of the NINDB on headache.

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Long-latency cerebellar responses in cerebellar pedunculi and cortex

Shigeyuki Deura, M.D.

ALTHOUGH SOME INFORMATION is available concerning the short- and long-latency cerebellar evoked potentials induced by cerebral cortical stimulation,[1–3] complete understanding concerning their origin has not been established. Recently, Jansen[4] clearly separated and systematically analyzed these responses and came to the conclusion that the short-latency response represented activation of granular cells of the cerebellar cortex and the long-latency one represented that of Purkinje cells.

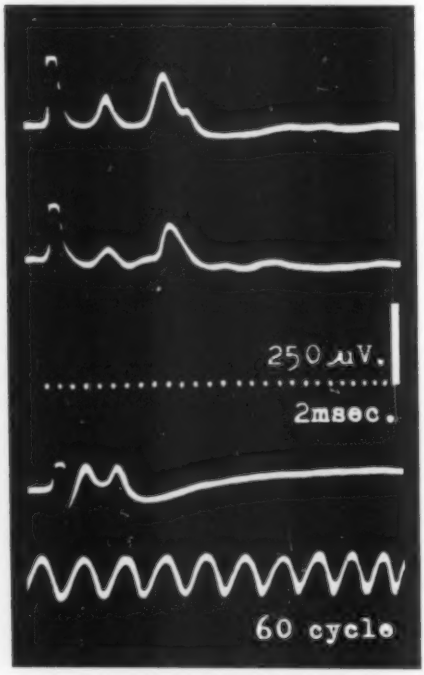

Fig. 1. Cerebellar responses in lobus anterior induced by stimulation of contralateral anterior sigmoid gyrus. Stimulus is indicated by first deflection (flat top), 10 volts (1 msec. duration) in top record and 7 volts (1 msec. duration) in next. Upward deflection represents positivity. All 3 traces of evoked responses were taken at same amplification; however, lower one utilized a slower sweep time.

In accordance with Jansen's division, the present electroanatomic study was undertaken to investigate cerebrocerebellar interaction indicated by these two types of responses. Pathways of the cerebellipetal impulses by which activation of Purkinje cells would be induced were also explored by observing the changes in cerebral stimulation required to evoke the cerebellar action potentials after cutting of the cerebellar pedunculi.

MATERIAL AND METHODS

The present report is based on observations obtained in work on 28 adult cats. They were anesthetized with pentobarbital sodium (25 mg. per kilogram of body weight) given intraperitoneally. The posterior pole of one cerebral hemisphere was ablated, and the anterior lobe of that side and the posterior lobes of both sides of the cerebellum were exposed and explored. Stimulations were carried out by single square wave pulses, the duration (0.5 to 5 msec.) and strength (0.2 to 40 volts) of which could be altered independently within the limits stated. Concentric or paired silver wire (2 mm. separation) stimulating electrodes were used. They were lightly applied to the pial surface of various areas of the cerebral

From the Department of Anatomy, Northwestern University, Chicago, and the Department of Anatomy, Kyoto University, Japan.

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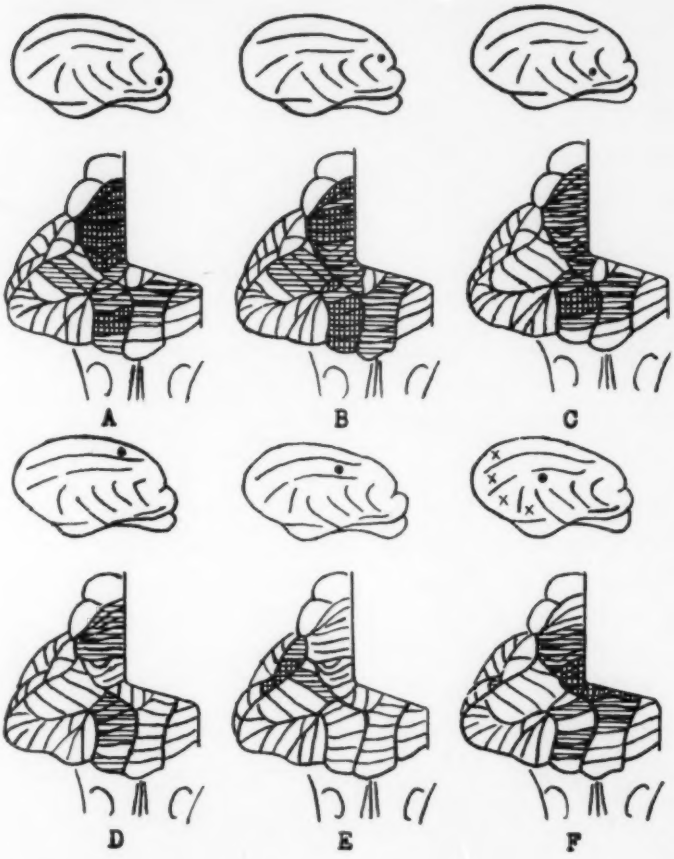

Fig. 2. Cerebellar responsive areas for corresponding cerebral cortical stimulation. Horizontally hatched areas indicate folia from which short-latency responses are recorded, and vertically hatched ones indicate where long-latency responses are observed. Crosses indicate cerebral areas whose stimulation does not induce cerebellar responses.

cortex. The time interval between 2 stimuli was never less than ten seconds.

The input electrode consisted of a silver wire 100 μ in diameter. An indifferent electrode was attached to the periosteum on the skull. Alterations in electrical activity were determined by a 4-stage push-pull amplifier and recorded on a 5-in. twin beam cathode ray oscillograph from which the amplified responses were photographed. In the records presented, upward deflections indicate positive potentials at the tip of the lead electrode.

Superior cerebellar pedunculi were cut after gentle caudalward displacement of the cerebellum. The middle and inferior pedunculi were cut from a lateral approach after upward displacement of the cerebellum. Following experimental use, animals were sacrificed. The brain was fixed in 10% formalin and embedded in celloidin; 40 μ frontal serial sections were made and stained by means of Weigert-Pal's stain. The completeness of the peduncle transection was checked histologically.

RESULTS

Cerebrocerebellar connections. Cerebellar action potentials were observed after electrical stimulation of various areas of the contralateral cerebral hemisphere. Figure 1 shows the result of a "typical" experiment in which the anterior

sigmoid gyrus was stimulated and electrical activity was recorded in the lobus anterior of the cerebellum. Only 2 types of cerebellar responses were recorded. The short-latency responses were positive monophasic and had a latency of 6 msec. (beginning of rising phase) and a duration of 4 to 5 msec.; the long-latency responses were positive-negative and had a latency of 15 msec. and variable duration depending on the negative component. While there occurred a shortening of the latency and a complication of the wave form in the long-latency responses as the strength of the shock was further increased above threshold, the short-latency responses were not altered even by the strongest stimuli used in these experimental conditions once stable recording conditions were reached.

Responses recorded from various parts of the cerebellar cortex could be divided into short- and long-latency ones. Figure 2 shows the responsive areas for corresponding cerebral cortical stimulation. In order to simplify the presentation, each cerebellar folium was repre-

TABLE 1
CEREBELLAR RESPONSIVE AREAS AND CORRESPONDING CEREBRAL STIMULATED REGIONS

Stimulated regions	*Responsive areas*	
	Long-latency response	*Short-latency response*
Gyrus sigmoideus anterior	Lobus anterior Lobulus simplex Lobulus paramedianus	Lobus anterior Lobulus simplex Tuber vermis Pyramis Lobulus paramedianus Crus I lobuli ansiformis Lobulus paramedianus (ipsilateral)
Gyrus sigmoideus posterior	Lobus anterior Lobulus paramedianus	Lobus anterior Lobulus simplex Tuber vermis Pyramis Lobulus paramedianus Crus I lobuli ansiformis
Gyrus lateralis anterior	——	Lobus anterior Lobulus paramedianus
Gyrus suprasylvius anterior	Lobulus paramedianus	Lobus anterior Lobulus simplex Tuber vermis Pyramis Lobulus paramedianus
Gyrus suprasylvius medius	Lobulus paraflocculari s	Crus I lobuli ansiformis Lobulus paraflocculari s
Gyrus ectosylvius medius	Lobulus simplex Tuber vermis	Lobus anterior Lobulus simplex Tuber vermis Pyramis Lobulus paramedianus Lobulus paraflocculari s
Gyrus lateralis posterior	——	——
Gyrus suprasylvius posterior	——	——
Gyrus ectosylvius posterior	——	——
Gyrus sylvius posterior	——	——

sented by a lead applied at its center. The horizontally hatched parts indicated the folia from which the short-latency responses were observed. These responsive areas and corresponding cerebral stimulated points are listed in Table 1.

The long-latency responses were obtained in the lobus anterior subsequent to stimulation of the anterior sigmoid and posterior sigmoid gyri. They were observed in the lobulus simplex from a stimulus at the anterior sigmoid and middle ectosylvian gyri, in the lobulus paramedianus from the anterior sigmoid, posterior sigmoid, and anterior suprasylvian gyri, and in the tuber vermis from the middle ectosylvian gyrus. On the other hand, the short-latency responses were present more widely. They were recorded in the lobus anterior after stimulation of the anterior sigmoid, posterior sigmoid, anterior suprasylvian, and middle suprasylvian gyri. Responses were obtained in the lobulus paramedianus from a stimulus at the anterior sigmoid, posterior sigmoid, anterior lateral, anterior suprasylvian, and middle ectosylvian gyri, in the crus I lobuli ansiformis from the anterior sigmoid, posterior sigmoid, and middle suprasylvian gyri, and in the ipsilateral lobulus paramedianus from the anterior sigmoid gyrus. Both short- and long-latency responses found in the lobulus parafloccularis were obtained as a result of stimulation of the middle suprasylvian gyrus. Action potentials were never observed in any part of the cerebellum by stimulation of the posterior lateral, posterior suprasylvian, posterior ectosylvian, and posterior sylvian gyri.

In these data, it was regularly observed that long-latency responses were recorded in the relatively restricted parts of the cerebellar cortex. As a result of this conclusion, cerebrocerebellar connections were investigated in more detail using these long-latency responses as indications. This time, the anterior lobe recording sites were divided into three longitudinal zones—zone medial to paravermal vein, zone adjacent to it, and zone lateral to it. The paramedian lobule was also divided into medial and lateral zones, and responses were recorded from the central area of each zone. In the other parts of the cerebellum, random sampling of electrical activity was carried out.

In Figure 3 are shown the results of stimulation in 5 cerebral areas and the responding areas of the cerebellum resulting therefrom. Dotted areas show the parts where the responses resulting from stimulation of just above threshold strength were observed (usually elicited by increasing voltage from 0.5 to 2 volts). These cerebrocerebellar relationships are summarized in Table 2.

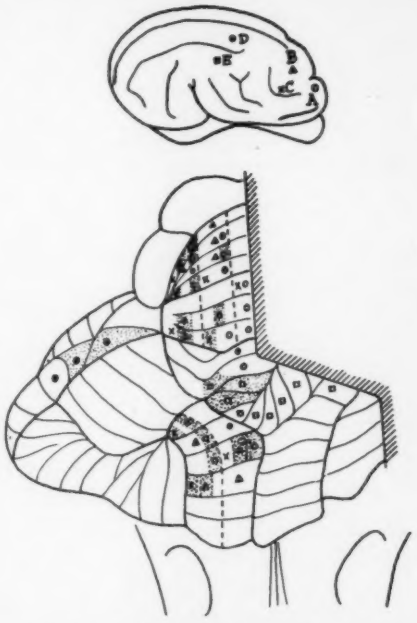

Fig. 3. Cerebrocerebellar connections indicated by long-latency responses. Dotted areas show responsive zones for just above threshold stimuli. These data are further summarized in Table 2.

While action potentials were never observed in the lobus anterior or lobulus paramedianus subsequent to stimulation of the middle supra- and middle ectosylvian gyrus, they appeared in the anterior part of the lobulus parafloccularis after stimulation of the middle suprasylvian gyrus and in the tuber vermis from the middle ectosylvian gyrus.

Since the cerebellar somatic receiving areas are located in the lobus anterior and lobulus paramedianus, somatotopic localization in the cerebrocerebellar connections were investigat-

ed. Location of the cerebral arm and leg somatic sensory areas were determined by stimulation of skin nerves in the fore- and hindlimbs, then long-latency responses were recorded in the lobus anterior and lobulus paramedianus when points within these 2 topographic subdivisions of the cerebral cortex were stimulated.

The upper 2 beams of Figure 4 show responses obtained from the same point of the lobulus paramedianus as a result of stimulation of the cerebral arm and leg areas, and the lower 2 indicate those obtained in the lobus anterior from stimulation of the same points. The cerebellar lobes receiving the action potentials were the same regardless of which topographic subdivision was stimulated, and there were no differences in the latency or wave form of the responses as long as the potentials were recorded from the same point.

It is widely accepted that the cerebellum consists of archi-, paleo-, and neocerebellar parts. Lobus anterior, which belongs to the paleocerebellum, and lobulus paramedianus, which belongs to the neocerebellum, were the parts in which long-latency responses resulting from cerebral stimulation were the largest and the most readily obtained. Comparison of the responses in these phylogenetically different parts was done in Figure 5. Responses in the

TABLE 2
CEREBROCEREBELLAR CONNECTIONS INDICATED BY LONG-LATENCY RESPONSES

Stimulated regions	*Responsive areas*	*Responsive areas for just above threshold stimuli*
Gyrus sigmoideus anterior	Lobus anterior medial posterior middle anterior posterior lateral anterior Lobulus simplex Lobulus paramedianus medial anterior middle lateral anterior middle	Lobus anterior middle anterior lateral anterior Lobulus paramedianus medial middle lateral anterior middle
Gyrus sigmoideus posterior	Lobus anterior middle anterior posterior lateral anterior posterior Lobulus paramedianus medial middle lateral anterior middle	Lobus anterior middle posterior lateral anterior posterior Lobulus paramedianus medial middle lateral middle
Gyrus coronarius	Lobus anterior medial posterior middle anterior posterior lateral anterior posterior Lobulus paramedianus medial middle lateral anterior middle	Lobus anterior middle posterior lateral anterior Lobulus paramedianus lateral anterior middle
Gyrus suprasylvius medius	Lobulus paraflocularis anterior	Lobulus paraflocularis anterior
Gyrus ectosylvius medius	Lobulus simplex posterior	Lobulus simplex posterior
	Tuber vermis	Tuber vermis

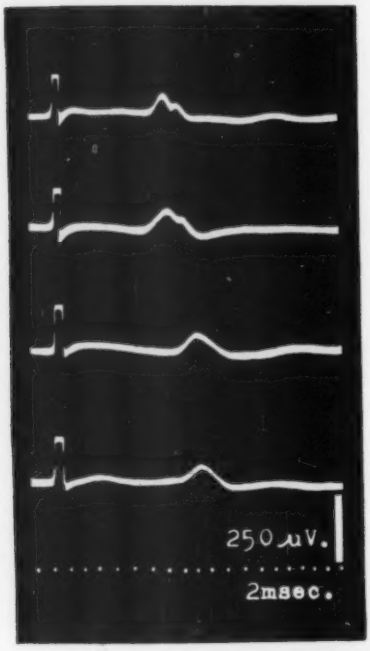

Fig. 4. Cerebellar responses evoked by stimulation of points within topographic subdivisions in the cerebral cortex. Pentobarbital sodium anesthesia. Stimulus is 4 volts (1 msec. duration) in all records.

1: Stimulation—sensory arm area. Record—lobulus paramedianus
2: Stimulation—sensory leg area. Record—lobulus paramedianus
3: Stimulation—sensory arm area. Record—lobus anterior
4: Stimulation—sensory leg area. Record—lobus anterior

lobus anterior showed a latency of 15 msec. and a threshold of 4 volts (1 msec. stimulus duration), and those in the lobulus paramedianus had a latency of 12 msec. and a threshold of 6 volts. Shortening of the latency, complication of the wave form, and the appearance and enhancement of the negative phase were observed in each of these responses as a result of stronger stimuli.

Pathway of cerebellipetal impulses. Since the cerebellar action potentials evoked by the cerebral cortical stimulation were activated by the impulses conducted via cerebellar pedunculi, pathways of the cerebellipetal impulses were investigated. Changes in the cerebral stimulation required to evoke the cerebellar responses were observed after cutting of the cerebellar pedunculi.

Figure 6 shows the changes of the evoked potentials after sectioning of the superior and inferior cerebellar pedunculi, and Figure 7 indicates the injured regions when viewed histologically. The long-latency responses in the lobus anterior and lobulus paramedianus were reduced in size after cutting of the superior pedunculi (Figs. 6a and 7, upper left) and never recovered. They disappeared completely when the middle pedunculi were cut (Fig. 7,

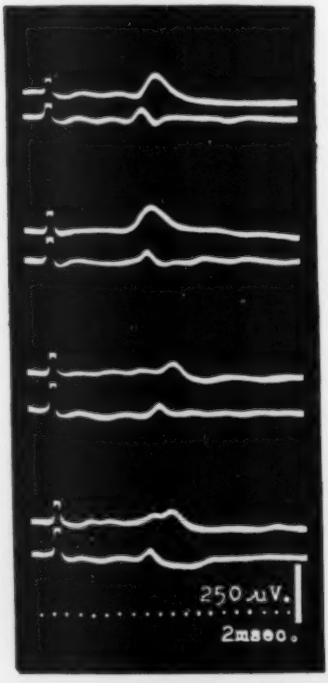

Fig. 5. Comparison of responses in lobus anterior and lobulus paramedianus. Upper beams of each record indicate responses in lobus anterior and lower ones indicate those in lobulus paramedianus. Pentobarbital sodium anesthesia. Stimulus is 6 volts (1 msec. duration) in all records.

upper right). While responses in the lobulus paramedianus reappeared twenty minutes after cutting of the inferior pedunculi (Figs. 6b and 7, lower left), those in the lobus anterior never recovered. Changes of the threshold of the cerebral stimulation required to evoke the cerebellar long-latency responses before and after the sectioning of the cerebellar pedunculi are listed in Table 3.

The threshold increased to 150% in the lobus anterior and to 112.5% in the lobulus paramedianus after section of the superior pedunculi. It increased to more than 750% in the lobus anterior and to more than 240% in the lobulus paramedianus after cutting of the inferior pedunculi. In the combined cuttings, threshold increased to infinity in those preparations accompanied by the cutting of the middle pedunculi and increased to infinity in the lobus anterior and to more than 240% in the lobulus paramedianus by the combined cutting of the superior and inferior pedunculi.

DISCUSSION

In the above mentioned experimental results, it was found that the cerebellar evoked potentials induced by stimulation of the cerebral cortex were divided into short- and long-latency responses and that the cerebrocerebellar connections indicated by the long-latency responses were more restricted than those indicated by the short-latency responses.

Two kinds of cerebellar responses were observed previously by Dow,[1] Hampson, Harrison, and Woolsey,[2] Stoiber,[3] and Jansen,[4] among whom Jansen clearly separated and systematically analyzed these responses and came to the conclusion that the short-latency responses represented activation of granular cells of the cerebellar cortex and the long-latency ones represented activation of Purkinje cells.

It is difficult to interpret these data in the light of Jansen's[4] report. Nevertheless, it would appear that the most logical interpretation of the long- and short-latency responses would involve 2 pathways possessing either different rates of conduction or different synaptic combinations. The classic cerebropontocerebellar system is known to be involved.[4] At least 3 possibilities exist for the second pathway: [1] the corticoolivocerebellar system,[5,6] [2] the corticoreticulocerebellar system (see Kuypers[7] for corticoreticular connections), or [3] the corticorubrocerebellar system.[8] It is beyond the scope of the present report to carry out a detailed study on the above mentioned extrapontine systems. The possibility of the second pathway involving the climbing fibers by way of the pontine nuclei was discussed by Jansen,[4] and he came to the conclusion that this was not likely in view of the anatomic study of Brodal and Jansen.[9] Snider[10] failed to find evidence of climbing fiber degeneration following large lesions which completely transected the pons. This would also argue against a direct ascending connection to the Purkinje cells.

Cerebrocerebellar connections indicated by

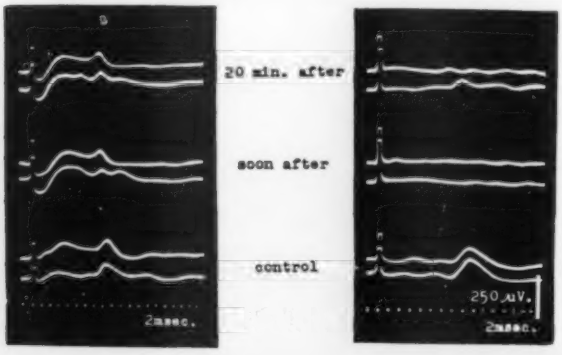

Fig. 6. Changes of cerebellar responses after cutting of cerebellar pedunculi. Upper beams of each record are responses in lobus anterior and lower ones are those in lobulus paramedianus. Pentobarbital sodium anesthesia. Stimulus is 6 volts (1 msec. duration).
[a] Cutting of superior cerebellar pedunculi
[b] Cutting of inferior cerebellar pedunculi

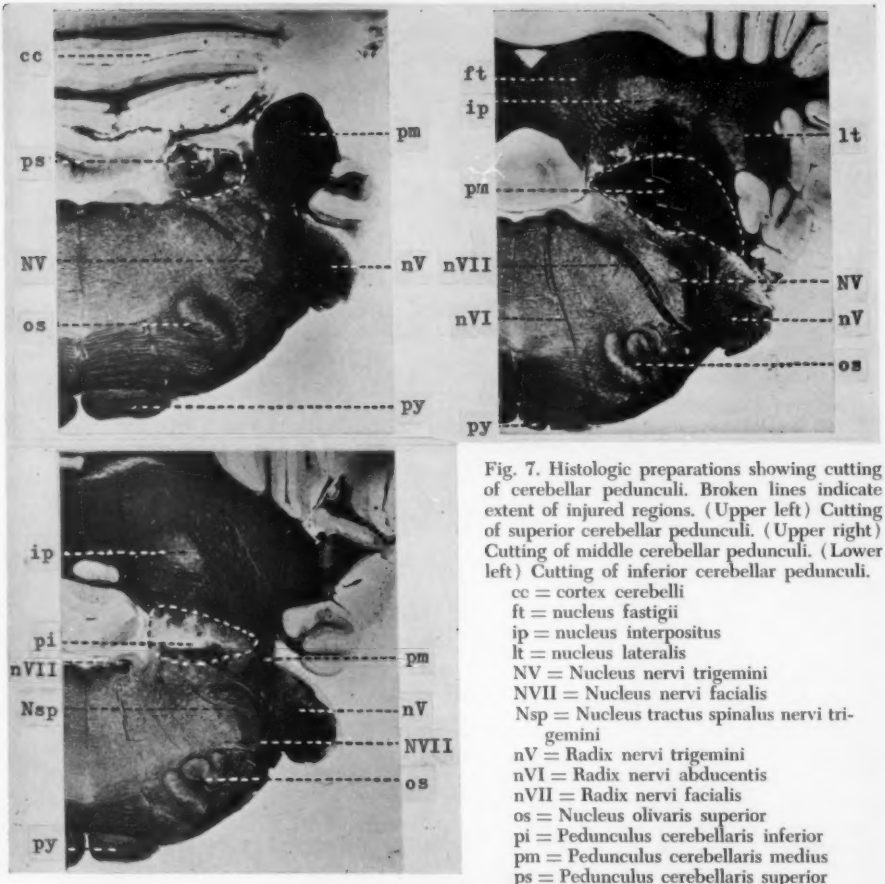

Fig. 7. Histologic preparations showing cutting of cerebellar pedunculi. Broken lines indicate extent of injured regions. (Upper left) Cutting of superior cerebellar pedunculi. (Upper right) Cutting of middle cerebellar pedunculi. (Lower left) Cutting of inferior cerebellar pedunculi.

cc = cortex cerebelli
ft = nucleus fastigii
ip = nucleus interpositus
lt = nucleus lateralis
NV = Nucleus nervi trigemini
NVII = Nucleus nervi facialis
Nsp = Nucleus tractus spinalus nervi trigemini
nV = Radix nervi trigemini
nVI = Radix nervi abducentis
nVII = Radix nervi facialis
os = Nucleus olivaris superior
pi = Pedunculus cerebellaris inferior
pm = Pedunculus cerebellaris medius
ps = Pedunculus cerebellaris superior
py = Tractus pyramidalis

the long-latency responses were not so restricted as were those observed by Snider and Eldred,[11] and the somatotopic localization between cerebral and cerebellar somatic receiving areas was not so clearly indicated in the lobus anterior[12] and lobulus paramedianus. But concerning the auditory (middle ectosylvian gyrus) and vestibular (anterior suprasylvian gyrus) systems, there seemed to be the same projections as indicated by these authors. Since Snider and Eldred used Chloralosane (30 to 40 mg. per kilogram of body weight) plus Nembutal (20 mg. per kilogram of body weight) and only pentobarbital sodium was used in the present study, some incompatibilities in the 2 groups of data can be expected.

On the basis of anatomic evidence, Jansen and Brodal[13] claimed that the cerebellum consists of 3 longitudinal zones: hemispheral, intermedial, and vermal. Chambers and Sprague[14,15] observed that Jansen and Brodal's concept would be applicable functionally and also pointed out that the lobus anterior was divided into a vermal-fastigial system and intermedial-interpositional system bordering on the paravermal vein. In the results of recording cerebellar action potentials from 3 longitudinally divided zones in the lobus anterior, no strict

TABLE 3
CHANGES OF THRESHOLD OF CEREBRAL STIMULATION AFTER SECTIONING OF CEREBELLAR PEDUNCULI

Case no.	Severed regions	Changes of threshold — Lobus anterior	Changes of threshold — Lobulus paramedianus
3	Pedunculi medius and inferior	Infinity	Infinity
4	Pedunculi inferior	750%	240%
5	Pedunculi superior	150%	112.5%
6	Pedunculi superior	150%	115%
10	Pedunculi superior and inferior	Infinity	240%
11	Pedunculi superior and medius	Infinity	Infinity
12	Pedunculi medius	Infinity	Infinity
13	Pedunculi superior and medius	Infinity	Infinity

localization to 1 of these longitudinal zones could be observed; as can be seen from Figure 3, the responses were widespread. Responses were also recorded from 2 longitudinally divided portions in the lobulus paramedianus, but no definit localization in the cerebrocerebellar connections was found under this experimental condition.

Action potentials were recorded in the lobulus paramedianus as a result of the stimulation of the anterior sigmoid, posterior sigmoid, anterior lateral, anterior suprasylvian, and middle ectosylvian gyri. These results agreed with Hampson's[16] and Snider and Eldred's[11] findings but contradicted those of Curtis.[17] Although Hampson and associates[2] have suggested that the anterior lobe was activated first and relayed to the posterior cerebellum, the fact that the latency of the cerebellar response was 3 msec. shorter in the lobulus paramedianus than in the lobus anterior indicates this was not the case. Furthermore, the fact that the responses disappeared in the lobus anterior while they reappeared in the lobulus paramedianus after sectioning of the inferior cerebellar pedunculi suggests that individual pathways could arrive at these 2 parts of the cerebellum from cerebral cortex perhaps involving 2 different synaptic systems. Since the threshold of the cerebral stimulation required to evoke cerebellar long-latency responses increased after section of the superior cerebellar pedunculi, cerebellipetal impulses may pass through here. Although anatomic evidences have pointed out that the superior pedunculi consist largely of cerebellifugal fibers, Ogawa[18] found that the tectocerebellar tract passes through the medial part, and Snider[19] recorded action potentials in the lobulus simplex and tuber vermis induced by stimulation of the superior colliculus.

Action potentials were never observed in the lobus anterior and lobulus paramedianus after cutting of the middle cerebellar pedunculi. It is suggested that the impulses traversing the middle pedunculi reached to both neo- and paleocerebellar cortices and had strong facilitatory actions.

Responses disappeared in the lobus anterior and reduced their sizes in the lobulus paramedianus after cutting of the inferior cerebellar pedunculi. Cerebellipetal impulses traversing the inferior pedunculi would reach to these portions and had stronger effect in the lobus anterior than the lobulus paramedianus. As can be seen from Table 3, cerebrocerebellar influences may be exerted through all 3 pairs of peduncles.

SUMMARY

The electroanatomic study was undertaken to investigate cerebrocerebellar interaction indicated by the short- and long-latency cerebellar evoked potentials and to explore the pathway of cerebellipetal impulses by which activation of Purkinje cells would be induced.

1. Cerebrocerebellar connections indicated by the short-latency responses were more widely distributed than were the long-latency ones. Action potentials in response to stimulation of the cerebral cortex were observed in the contralateral lobus anterior, lobulus simplex, tuber vermis, pyramis, lobulus paramedianus, crus I lobuli ansiformis, and lobulus paraflocculari s. Responses were present in the homolateral lobulus paramedianus occasionally. The short-latency responses were elicited after stimulation of the anterior sigmoid, posterior sigmoid, anterior lateral, anterior suprasylvian, middle suprasylvian, and middle ectosylvian gyri. No responses were seen after stimulation of the posterior lateral, posterior suprasylvian, posterior ectosylvian, and posterior sylvian gyri.

2. Cerebrocerebellar connections indicated

by the long-latency responses were relatively restricted. Action potentials, resulting from stimulation of the cerebral cortex, were present in the contralateral lobus anterior, lobulus simplex, tuber vermis, lobulus paramedianus, and lobulus paraflocculariis. Responses were elicited after stimulation of the anterior sigmoid, posterior sigmoid, coronal, middle suprasylvian, and middle ectosylvian gyri. In the results of recording long-latency responses from 3 longitudinally divided zones in the lobus anterior and 2 longitudinally divided zones in the lobulus paramedianus, no strict localization to 1 of these zones could be observed.

3. Somatotopic localization between cerebral and cerebellar somatic receiving areas was not clearly indicated in the lobus anterior and lobulus paramedianus. But concerning the auditory and vestibular systems, the cerebral auditory area projected to the cerebellar auditory area.

4. Thresholds of cerebral stimulation which evoke the cerebellar responses increased after section of the superior, middle, and inferior cerebellar pedunculi. Cerebellipetal impulses passing through these pedunculi could reach both neo- and paleocerebellum and provide firing of the cerebellar cortical cells. Impulses via middle pedunculi have the strongest influences.

The author wishes to express his sincere appreciation to Dr. Ray S. Snider for his assistance in this work.

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Cardiorespiratory syndrome of extreme obesity with papilledema

Report of a fatal case with electroencephalographic, metabolic, and necropsy studies

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THE HYPOVENTILATION or cardiorespiratory syndrome due to extreme obesity (also known as the pickwickian syndrome) was first recognized as a clinical entity in 1936.[1–5] The syndrome consists of extreme adiposity, diminished vital capacity, polycythemia, and cyanosis. Neurologic symptoms usually accompany the disorder but have not been adequately studied or described. In the few proved cases reported so far, neurologic symptoms have included apraxia of gait, drowsiness, signs of frontal lobe disturbance, and electroencephalographic abnormality.[6] The present communication describes a proved case in which the following neurologic symptoms and signs were present: extreme drowsiness, headache, papilledema, unsteady gait, myoclonic jerks, irregular respiration, and death due to respiratory failure. The diagnosis was confirmed by necropsy study of the brain.

Although papilledema has been noted in association with pulmonary insufficiency due to emphysema, as far as we know this is the first proved case of hypoventilation due to extreme obesity with papilledema. Although the diagnosis was recognized and established clinically, there were many features simulating hypothalamic tumor. The neurologic complications of extreme obesity are extremely serious, and other cases have proved fatal.[4] In the present case, pulmonary hypoventilation with cerebral anoxemia and CO_2 retention was proved by metabolic studies during life; serial electroencephalograms were made, and the diagnosis was established by arteriograms and pneumoencephalogram and was later confirmed at necropsy.

CASE REPORT

A 29-year-old Negro woman was admitted to the Neurology Service at Detroit Receiving Hospital because of headache increasing in frequency and severity for six months. Obesity and drowsiness had been present for two years, but dyspnea, orthopnea, and an enormous gain in weight had occurred during the six months before admission.

Birth and developmental history were normal except that she had been obese all her life. She left school after completing the ninth grade. She denied any other illnesses except pelvic inflammatory disease which was successfully treated at this hospital five years before. Six years earlier, she had a normal pregnancy. Social and family history revealed nothing remarkable.

The present illness began two years before admission with drowsiness similar to narcolepsy, except that she could stay awake by "concentrating hard." The periods of sleep increased, and, during the previous six months, she slept most of the day (as much as twenty

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to twenty-three hours). Sleep regularly occurred if she relaxed after her household duties but did not occur when she was talking or while she was walking. She had no catalepsy or hypnagogic hallucinations. Her weight had increased from 240 lb. to a maximum of 330 lb. immediately before admission. Progressive dyspnea had been present for one year. Eventually, she was unable to walk more than 50 yd. because of shortness of breath. For six months, she was unable to lie flat because of orthopnea and began to complain of dull, mild frontal headaches, worse toward the end of the day, occurring about three times a week, and lasting two to four hours.

Inquiry revealed a daily dietary intake of about 4,000 to 5,000 calories. The diet was well balanced, and she denied excessive ingestion of salt or the use of any drugs. She had some frequency of urination but no incontinence, polyuria, or polydypsia. Menarche began at 17 years of age, and menses had been of the usual duration and continued through her hospital admission. The patient denied convulsions, paralysis, or disorders of sensation.

Fig. 1. Clinical photograph (obtained with consent) of patient while in hospital for treatment of cardiorespiratory syndrome of extreme obesity with papilledema. At time of photograph, patient was dyspneic, orthopneic, and narcoleptic and showed myoclonic jerks of small muscles of hands.

Physical examination. The patient was a tremendously obese woman (Fig. 1) in acute distress, dyspneic at rest but particularly after exercise. She was 5 ft., ½ in. tall and weighed 340 lb. The pulse was 70 per minute and regular, and the blood pressure was 120/70. There was black cyanosis of the nail beds but no clubbing. The patient fell asleep several times during the examination but could be aroused and, if given a specific task, was able to concentrate on it and keep awake. Rapid, shallow, periodic breathing of the Biot type was noted with occasional Cheyne-Stokes respiration. Percussion dullness was heard at both bases to the seventh thoracic vertebra, and, by percussion, the diaphragm was elevated bilaterally with some displacement of the heart to the left. No murmurs were heard. There was no edema. Abdominal examination revealed no tenderness or masses. Pelvic and rectal examinations were negative. Measurements taken of the patient on admission were as follows: chest, 63 in.; waist, 65 in.; hips, 70 in.; midthigh, 26 in.; upper arm, 12½ in.

Neurologic examination. The patient was a drowsy woman who was oriented to person, place, and time but who lapsed repeatedly into sleep. The gait was short-stepped, tremulous, and unsteady. She was of average intelligence and memory; judgment and affect were within normal limits. Speech was staccato because of severe dyspnea but was not dysarthric. Visual acuity was 20/20 bilaterally, and the visual fields were full to confrontation and upon examination by tangent screen. Slight enlargement of the physiologic blind spot was noted bilaterally. The fundi revealed bilateral papilledema of 4 diopters, with considerable venous engorgement. Some watery exudate with perivascular streaking and a few early hemorrhages around the veins at their entry into the optic nerve were noted. The veins were blue-black, tortuous, and dilated. Other cranial nerves were intact. Strength and coordination were normal. Irregular, twitching movements of parts of the muscles of the arms and hands were noted bilaterally, particularly when the patient was asleep. The muscular jerks were myoclonic and of small amplitude but were not precipitated by loud sounds or tactile stimulation. The tendon jerks in the arms were equal but brisk, and there was a

positive Hoffmann sign bilaterally. The abdominal reflexes could not be elicited. The tendon jerks in the legs were reduced symmetrically. There were bilateral extensor plantar reflexes. Bilateral grasp reflexes were present, but there was no sucking or glabellar reflex present. Sensory examination was normal.

Laboratory examination. Urine examination showed no abnormalities. The hemoglobin was 16.8 gm. with an erythrocyte count of 5,900,000 per cubic millimeter. Leukocyte count was 7,000 per cubic millimeter with a normal differential count. The serologic test for syphilis was negative. Total blood cholesterol was 204 mg. per cent. Serum protein was 7.2 gm. with 4.1 gm. of albumin and 3.1 gm. of globulin. Plasma glucose was 90 mg. per cent. Prothrombin time was normal. Serum calcium was 4.7 mEq. per liter. Urine output over twenty-four hours was not increased.

Bilateral carotid arteriography revealed no abnormalities. A lumbar puncture revealed an opening pressure of 480 mm. of water and a closing pressure of 460 mm. of water. The fluid was clear, without cells, and total protein was 20 mg. per cent. Venous pressure was 300 mm. of saline above the sternal level and the circulation time (arm to tongue with Decholin) was seventeen seconds. Chest roentgenograms showed no cardiac enlargement or pulmonary congestion. An electroencephalogram done on admission revealed an abnormal pattern with generalized theta activity diffusely but more noticeable in the left temporal region. Serial electroencephalograms were obtained and showed progressive worsening (Fig. 2). Pulmonary function studies were kindly performed by Dr. Benjamin Lewis of the Department of Medicine at Detroit Receiving Hospital and revealed the following: Vital capacity was 1,365 cc. (calculated normal: 2,400 cc.). The timed vital capacity was 86% in one minute. The maximum breathing capacity was 14.1 and 22.8 liters per minute (calculated normal: 92 liters per minute). Maximum expiration velocity was 55.1 liters per minute (calculated normal: 400 liters per minute). Arterial oxygen saturation at the time of these studies was 85.8% but was lower at a later date. The arterial *p*H was 7.30 units and pCO_2 was 63.7 mm. (normal: 35 to 40 mm.). An electrocardiogram revealed right bundle branch block. Combined ventriculogram and pneumoencephalogram performed by Dr. Harold Portnoy revealed a small ventricular system without displacement and no evidence of tumor.

Treatment consisted of oxygen administered by nasal catheter slowly at 1 liter per minute intermittently, moderate dehydration, and strict reducing diet (800 calories per day). Medications consisted of Hydrodiuril, oral sodium bicarbonate (900 mg. daily), Ritalin (30 mg. daily), and penicillin (1,200,000 units daily). An organic buffer (Talatrol, TRIS, or trishydroxymethylaminomethane) was given intravenously to reduce the elevated plasma CO_2.[7,8] It was administered in doses of 36.3 gm. with 1.75 gm. of sodium chloride and 0.37 gm. of potassium chloride in 1,000 cc. of water each day for three days. A reduction in the CO_2 level in the blood was noted after each treatment. Although the clinical diagnosis was hypoventilation syndrome of extreme obesity with anoxemia and hypercapnia, with resultant pseudotumor cerebri, it was considered necessary to confirm the diagnosis by the pulmonary function studies cited and to exclude hypothalamic tumor by arteriogram and ventriculogram.

Course in hospital. Carotid arteriograms were performed two days after admission. Right carotid injection was accomplished without difficulty. However, injection of the left carotid artery was difficult due to her obesity and the thickness of her neck. Some dye was unfortunately injected into the carotid sheath. No complication was noted until two hours after the procedure, when the patient complained of weakness of the right arm and leg and examination revealed flaccid right hemiparesis which improved after thirty minutes. Presumably, an embolus had resulted from thrombosis at the injury to the left carotid artery during injection. This deficit improved rapidly, and, after four days, only slight weakness of the leg remained. Drowsiness and dyspnea improved with treatment. Weight was reduced by 1 lb. each day during the first nineteen days, and she became much less dyspneic and more alert. Ambulation was begun, and she was anxious to go home. At 2 A.M. on the morning of the twenty-fifth hospital day, a nurse noted she was orthopneic;

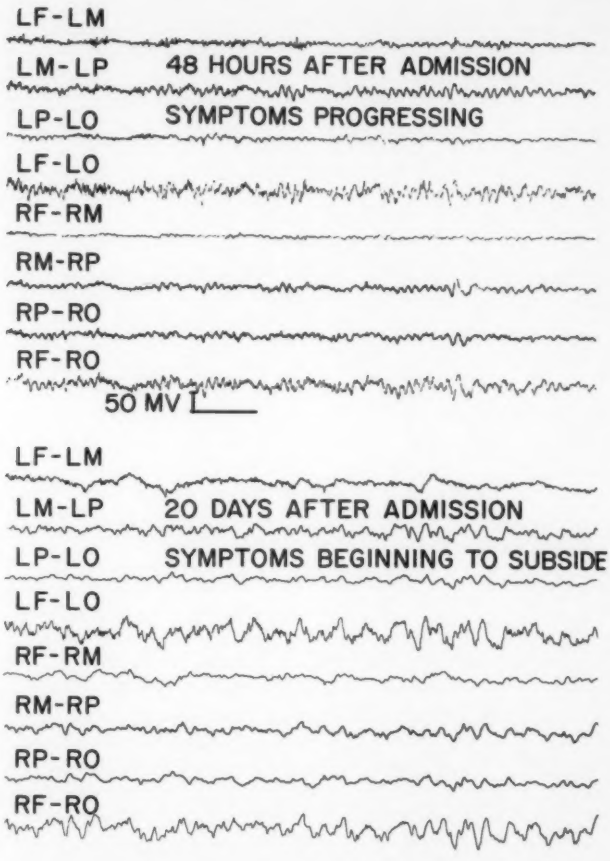

Fig. 2. Samples of electroencephalographic records made three days (upper record) after admission and three weeks after admission (lower record) to show theta and delta slow activity. High voltage theta and delta activity is compatible with cerebral anoxia. Lower voltage irregular slowing is compatible with severe hypercapnia.[25]

100% oxygen was given at 7 liters per minute for two hours, and respirations ceased shortly thereafter. When the neurology resident arrived, respirations and cardiac function had stopped and the patient could not be revived. Apnea was almost certainly due to respiratory depression resulting from oxygen therapy, which is a well-known therapeutic hazard in hypercapnia.

Cerebral metabolic studies. *Methods.* When the patient appeared to be improving, simultaneous determinations of arterial, femoral venous, and jugular venous blood gases and electrolytes were made.

Cerebrospinal fluid, *p*H, sodium, potassium, and pCO_2 determinations were also obtained (Tables 1 and 2). Due to technical error, flame photometric determinations of cerebrospinal fluid sodium and potassium were not obtained at the same time as the blood samples (since oil had been added to the spinal fluid), but these determinations were done on cerebrospinal fluid obtained four days later.

Blood oxygen and carbon dioxide content were determined by the manometric technic of Van Slyke and Neill.[9] The *p*H of blood and cerebrospinal fluid were measured with a Radiometer 22 *p*H meter and a Beckman blood *p*H electrode assembly. Blood pO_2 was calculated from the *p*H and the oxyhemoglobin saturation, using the dissociation curves of Dill.[10] Arterial pCO_2 was calculated from *p*H, CO_2

content, and hematocrit, by means of the nomogram of Singer and Hastings.[11] Spinal fluid pCO_2 was calculated by means of the Henderson-Hasselbalch equation using a pK of 6.13. Sodium and potassium concentrations of blood and cerebrospinal fluid were obtained by a flame photometer. Sodium activity was measured with a sodium electrode (NAS_{11-18}) and a Radiometer *p*H meter.[12]

Results of the cerebral metabolic studies are presented in Tables 1 and 2. Arterial oxygen saturation was 68%, pO_2 was 39 mm. Hg, pCO_2 was 54 mm. Hg, and *p*H was 7.35 units. Jugular venous oxygen saturation and pO_2 were both extremely low. Femoral venous pO_2 was decreased, and pCO_2 was increased but less so than in cerebral venous blood. Cerebral arteriovenous oxygen (AVO_2) difference was slightly increased but within normal limits. The cerebral respiratory quotient was depressed. Both *p*H and pCO_2 of cerebrospinal fluid were lower than those of arterial blood. Sodium activity of the cerebrospinal fluid was higher than simultaneous determinations of blood values. Cerebral arteriovenous differences for both sodium activity and concentration were negative; difference for potassium concentration was positive. Sodium and potassium concentrations for cerebrospinal fluid drawn four days after the other samples were taken were lower than blood values.

Necropsy studies. Necropsy was limited to the brain. The skull was thickened, showing hyperostosis frontalis interna. Increased calcification and bony density also were noted in both petrous ridges. The middle ear was opened and found to be within normal limits. The dura was unremarkable; the dural sinuses were all patent. The brain was examined first in situ and showed dense congestion of the cortical vessels, all the veins being distended and enlarged and filled with liquid blood. The brain itself (after removal and emptying of the vessels) was small. No palpable softening and no evidence of herniation at the falx, foramen magnum, or tentorial edge were found. The brain was fixed and hardened in 10% formalin. It was then sectioned in coronal sections. Cut sections of the brain showed an unusual congestion and vascular engorgement diffusely through the brain. The cortex appeared unusually dark. There was no evidence of significant atherosclerosis. The circle of Willis was normally formed. The white matter was

TABLE 1
RESULTS OF CEREBRAL METABOLIC STUDIES

	O_2 content	*pO_2*	*CO_2 content*	*pCO_2*	*pH*	*O_2 saturation*	*Respiratory quotient*
Arterial	15.04 (19.6)*	39.4 (94)	54.12 (48.2)	54.5 (40)	7.35 (7.4)	68	
Jugular venous	8.24	22.7	60.26	63.0	7.33	38	
Femoral venous	11.15 (12.9)	28.1 (40)	57.22 (54.8)	58.0 (46)	7.35	51	
Cerebral arteriovenous	+6.80 (63)†	+16.7	−6.14	−8.5	+0.02	30	0.9 (0.99)†
Cerebrospinal fluid			58.32	50	7.34		

*Normal values are placed in parentheses after values obtained in patient. Normal values cited from *Handbook of Respiratory Data in Aviation.*[10]
†Normal values cited from Kety and Schmidt.[19]

TABLE 2
RESULTS OF ELECTROLYTE STUDIES

	Sodium (*mEq. per liter*)	*Potassium* (*mEq. per liter*)	*Sodium ionic activity* (*mV*)
Arterial	138	3.7	77.5
Jugular venous	140	3.5	78.5
Femoral venous	144	3.7	75.0
Cerebral arteriovenous	−2	+0.2	−1.0
Cerebrospinal fluid	137*	3.0*	79.0

*These samples were drawn four days after all other samples.

unremarkable except for the ventricular needle punctures which were visible entering the lateral ventricles. A small infarct approximately three weeks old was found in the putamen and appeared by history to be a complication of the arteriogram. The ventricles were small. The brain weighed 1,050 gm. and was small in size in spite of the marked vascular congestion. Microscopic sections were made.

Microscopic examination by Professor J. L. Chason confirmed the opinion of a three-week-old anemic infarct. Moderate anoxic changes were noted along with increased vascular engorgement. A small glial nodule was noted in the right side of the medulla in the area of the lateral reticular substance, and a recent anemic infarct of microscopic size was seen on the opposite side. The pituitary gland was removed and appeared normal. The pituitary weighed 1.2 gm., and nothing unusual was noted grossly or microscopically.

DISCUSSION

Cardinal features of the cardiorespiratory syndrome of extreme obesity are obesity, cyanosis, arterial oxygen undersaturation, and polycythemia. Carbon dioxide retention may also occur as shown by the present case. The primary cause of the disorder is pulmonary hypoventilation. In the present case, the vital and maximum breathing capacities were reduced, accounting for the anoxemia, and the gross reduction of the expiratory velocity accounts for the CO_2 retention. No emphysema was present. Cardiac disorder also plays a part. The pulmonary hypoventilation is due to extra work necessitated to move the fat-laden abdomen and thorax, encroachment on the thoracic space by upward displacement of the diaphragm, and increased mediastinal fat.[2] The response to CO_2 as a respiratory stimulus is often decreased, and, in all obese subjects, the work, ventilation, and oxygen cost of breathing are increased proportionately with the weight.[13] Whether increased mediastinal fat itself impairs cardiac function is not known. Complete cure may be achieved by weight reduction.

Decreased arterial oxygen saturation accounts for the polycythemia and previously has been assumed to account for the cerebral symptomatology and right cardiac ventricular failure, presumably as a result of pulmonary arterial hypertension which has been shown to occur by cardiac catheterization.[14] This accounts for the prolonged circulation time, increased venous pressure, and right bundle branch block.

Our patient is of unusual interest because CO_2 retention occurred similar to that occasionally seen in severe emphysema and appeared to be responsible for the papilledema, drowsiness, and muscular twitching. These signs are characteristic of CO_2 narcosis[15] rather than anoxemia. The fatal termination was probably due to the use of oxygen, which is known to cause respiratory arrest in CO_2 narcosis, since the respiratory center is driven by anoxia rather than CO_2. In addition, oxygen breathing causes liberation of CO_2 from erythrocytes.[16] It should be stressed that this is a serious metabolic disease with predominantly neurologic symptoms requiring emergency medical treatment. If CO_2 retention is marked, assisted mechanical respiration is required.[17,18] In retrospect, mechanical respiration should have been used in the patient presented here. Cerebral metabolic studies confirmed the clinical impression that a combination of severe arterial hypoxia and hypercapnia was present, although the cerebral A-VO_2 remained within the upper limits of normal values.

Reduction of arterial pO_2 or elevation of arterial pCO_2 produced by changing the composition of the inspired air has been shown to decrease cerebrovascular resistance and to increase cerebral blood flow. Carbon dioxide breathing produces a rapid increase of cerebrospinal fluid pressure due to increased intracranial blood volume.[19,20] Studies in this clinic of patients awake and asleep during constant recording of cerebrospinal fluid pressure show that the pressure rises further during sleep due to additional CO_2 retention. Changes in blood gas tensions similar to those reported in the present case have been determined in subjects with pulmonary emphysema and have been shown to be associated with increased cerebral blood flow, decreased cerebral oxygen consumption, and lowered cerebral A-VO_2.[21]

In the patient reported here, an increase of cerebral oxygen consumption is improbable in the presence of mental obtundity and drowsiness with slow activity in the electroencepha-

logram and a depressed cerebral respiratory quotient. Thus, in our patient, it is probable that cerebral blood flow was decreased, as judged by the slightly increased cerebral A-VO_2, despite marked cerebral congestion.

In the past, elevation of cerebrospinal fluid pressure in patients with anoxia and hypercapnia has been explained by the following possibilities: increased cerebrospinal fluid volume, cerebral edema, and increased intracranial blood volume due to intense vasodilation and venous engorgement.[22,23] In our patient, the increased cerebrospinal fluid pressure appeared to be due to increased intracranial blood volume, increased systemic venous pressure, and intense cerebral venous congestion, since the pneumoencephalogram showed small ventricles. At necropsy, brain tissue volume was small and there was no edema despite intense congestion. This view is supported by the facts that venous engorgement was present in the brain at necropsy, and, during life, the retinal veins were visibly distended with dark blue-black blood.

Westlake and Kaye[22] reported that, in CO_2 narcosis, there was a relationship between arterial *p*H, pCO_2, and mental state. Most patient were conscious if the *p*H was above 7.3 or the pCO_2 less than 80 mm. Hg. If one accepts this conclusion, the changes in arterial *p*H and pCO_2 in our patient were not sufficient to explain her depressed mental state. However, in an experimental study of CO_2 narcosis, we have demonstrated that changes in the electroencephalogram correlated better with the *p*H of brain tissue rather than with the arterial *p*H. [15,24] If the *p*H of the monkey cortex was reduced below 6.6 to 6.9 units, electroencephalograpic abnormality regularly resulted. Electroencephalographic changes correlated less well with brain tissue pCO_2 and poorly with arterial pCO_2 or *p*H. In our patient, a state of chronic cerebral anoxemia also existed, and, for reasons stated below, we believe that acidosis and hypercapnia of brain tissue were more severe than is reflected in values obtained from the blood and cerebrospinal fluid. In CO_2 narcosis, the electroencephalographic activity is of low amplitude and consists of irregular theta-delta slowing. The activity seen in the present patient was of this type, although anoxemia also probably played a part. Only in the late stages of anoxia does the electroencephalogram become of low voltage and irregular. Before this, anoxia causes hypersynchronous theta and delta activity of high amplitude.[25]

It has been reported in experiments with animals that the cerebrospinal fluid *p*H is significantly lower than the arterial *p*H and that spinal fluid pCO_2 is higher than arterial pCO_2.[26,27] Manfredi and associates[20] reported a case of papilledema with chronic respiratory acidosis due to pulmonary emphysema with studies of the blood and cerebrospinal fluid pCO_2. In this case, during respiratory acidosis, the arterial blood : cerebrospinal fluid *p*H gradient was negative and the corresponding blood : cerebrospinal fluid pCO_2 gradient was positive. When respiratory acidosis was corrected, both gradients returned to normal. In our patient, like that reported by Manfredi and associates, a positive arterial blood : spinal fluid pCO_2 gradient was observed. Because of technical difficulties in obtaining cerebrospinal fluid uncontaminated by exposure to air, which, unfortunately, is known to have occurred in the present case, further studies are needed before final conclusions can be drawn.

In normal individuals, the sodium concentration of cerebrospinal fluid is often found to be slightly higher than plasma sodium; hence, the higher sodium activity found in the spinal fluid compared to blood values in our patient is not unusual. In animal experiments, it is known that hypoxia and apnea cause only minor changes in the potassium concentration of cerebrospinal fluid and no measurable effect on sodium and calcium as determined by flame photometry.[28]

In our patient, cerebral arteriovenous sodium difference was negative and potassium difference was positive when measured by both flame photometry and ionic activity. The differences in ionic activity are significant and suggest the possibility of a sodium flux from brain tissue to blood and of a potassium flux in the opposite direction. Recently, we have shown that, during experimental cerebral hypoxia or hypercapnia or both with electroencephalographic slowing, there is a flux of sodium into the cortex and a flux of potassium into the extracellular space with an ionic flux in the opposite direction during recovery. From these

experimental data, it could be assumed that our patient was showing the flux of sodium and potassium associated with recovery from cerebral metabolic depression, in which sodium ions are actively transported out of brain tissue. We have made a systematic study of cerebral arteriovenous differences of sodium and potassium in the monkey, and such fluxes are demonstrable between brain and jugular venous blood in cerebral metabolic depression during hypoxia and ischemia.

Increased hydrogen ion concentration of blood and brain also tends to displace sodium and potassium ions into the extracellular space[25,29] before metabolic inhibition. Thus, acidosis causes initially increased sodium and potassium activity of the plasma and cerebrospinal fluid, and later anoxia causes a cellular sodium influx and potassium efflux with the reverse during recovery. That these phenomena occur in the erythrocyte as well as the nervous system has been shown by us. First, 30% CO_2 in oxygen and later 100% nitrogen were inhaled. Continuous records of sodium and potassium activity of the arterial blood during CO_2 inhalation showed increases of both sodium and potassium in the arterial blood as the arterial *p*H decreased. During nitrogen breathing, arterial sodium activity decreased and potassium increased as arterial oxygen saturation decreased. Then, 50 cc. of whole blood was removed and the 2 gas mixtures were bubbled through it separately with the same results. After centrifugation and removal of the erythrocytes, there was no change in sodium or potassium activity when the same gases were bubbled through the plasma alone. It was concluded that the erythrocytes appeared to be responsible for such cation fluxes occurring in the arterial blood. Such tests prove that cells must be present in the plasma for such cation changes to occur. Harris[30] has also discussed the evidence for a decreased flux of sodium into the erythrocyte with decrease in *p*H. Presumably, there are profound cation changes in peripheral nerve and muscle in acidosis and anoxemia as well as in erythrocytes and cortical neurons. Although spikes are occasionally seen in the electroencephalogram with CO_2 narcosis,[15] the myoclonic movements present in our patient appeared on clinical grounds to arise from excessive irritability of peripheral myoneural units. No convulsions occurred, and numerous electroencephalographic examinations failed to show spike activity when such myoclonic movements were present.

NATURE OF PAPILLEDEMA AND RELATION TO PSEUDOTUMOR CEREBRI

In this patient, it is clear that the papilledema was due to intense cerebral venous congestion resulting from a combination of cerebral anoxemia and hypercapnia. The frequent lapses into sleep probably depressed respiration further with increased arterial pCO_2 and further increase of intracranial pressure. Probably, the same mechanism is responsible for the papilledema seen in congestive heart failure and neurologic disorders associated with depressed respiratory exchange, such as poliomyelitis and postinfectious polyneuritis. We have seen two cases of papilledema, pseudotumor cerebri, and increased intracranial pressure after resection or accidental ligation of the internal jugular veins for radical neck dissection and suspect that this is due to hypercarbia limited to the cerebral circulation, resulting from the decreased cerebral capillary-venous flow.[15] Perhaps other cases of pseudotumor cerebri are due to diminished cerebral venous flow, which in turn is due to congenital or acquired venous disease. Certainly, this would seem to be the case in cortical thrombophlebitis and thrombosis of the dural sinuses. We are aware, however, that not all cases of pseudotumor cerebri are due to cerebral hypercarbia or venous circulatory disturbances. Pseudotumor cerebri with papilledema also occurs in vasculitis from viral and other conditions, from hypocalcemia, and from causes as yet unknown.

SUMMARY

An informative case is presented of the cardiorespiratory syndrome of extreme obesity with papilledema. The diagnosis was confirmed by pulmonary function and metabolic and necropsy studies. A combination of hypoxemia and hypercapnia was present. Lethargy, drowsiness, and myoclonic jerks of the fingers were noted. Papilledema appeared to be due to intense venous congestion of the brain without brain swelling resulting from

combined hypercapnia and anoxemia. The slow waves present in the electroencephalogram in this case appeared to be due to hypercapnia as well as hypoxemia. The possible relation of cerebral hypercarbia and diminished cerebral capillary-venous flow in some cases of pseudotumor cerebri is discussed.

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Fibrinolysis and the nervous system

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THE ROLE OF THE nervous system, both central and peripheral, in the activation of human fibrinolysis has scarcely been studied, though Macfarlane and Biggs[1] in 1946 demonstrated that anxiety induced fibrinolytic activity, and Kwaan and associates[2] in 1957 felt that a neurogenic mechanism was operative in clot dissolution. The purpose of this paper is to report the effects of a nonenzymatic drug, Pitressin, and a number of stressful neurologic procedures on the human fibrinolytic system. In addition, certain aspects of the relationship of the fibrinolytic system to neural function will be examined.

The treatment of thromboembolic states with thrombolytic agents has aroused much interest lately. In most preparations utilized, the active agent is the bacterial enzyme streptokinase. Therapy of cerebrovascular disease with these preparations now appears to be safe enough for clinical trial, though their effectiveness has yet to be convincingly demonstrated.[3] The large number of marked side effects obtained with the earlier products have, with the current purer materials, been reduced to a reasonable minimum.[4] However, there are still a number of objections to enzymatic therapy. The agents are rather expensive, and it is usually necessary to infuse large amounts of the streptokinase, particularly in those who possess high titers of antistreptokinase antibodies. These must be neutralized before the enzyme can effectively activate the fibrinolytic system. Streptokinase is highly antigenic, and retreatment after a few days or weeks has, in a significant number of cases, resulted in allergic or anaphylactic reactions.[5] Lastly, many investigators feel that arterial thromboses, which are more resistant to lysis than their venous counterparts, are best treated by direct instillation of the thrombolytic agent in the artery proximal to the site of occlusion.[6] This is an obvious inconvenience in the treatment of cerebral thromboses and emboli.

The fact that the human organism has a naturally occurring and probably constantly operative fibrinolytic system[7] has stimulated efforts to find nonenzymatic drugs which would produce endogenous activation. By thus causing a more physiologic thrombolysis than that induced by enzymatic drugs, a safer and more effective therapy might be found which might also help to clarify the fibrinolytic mechanism. In addition, clot lysis initiated in this manner might conceivably proceed despite the disappearance of induced fibrinolytic activity in the circulating blood. In vitro, this continuing lysis has been noted to occur with clots placed in brief contact with urokinase, a naturally occurring human fibrinolysis activator.[8] The list of nonenzymatic drugs that have been found to induce a fibrinolytic tendency in man is slowly growing, though not all of them produce a very strong or long-lasting lysis. Acetylcholine, epinephrine, Irgapyrin, Butazolidine, para-aminobenzoic acid, procaine, protamine, nicotinic acid, and protein-free pyrogens have been successfully used experimentally.[9] The latter two induce marked, but rather short, fibrinolytic activity. We discovered the ability of Pitressin to activate the fibrinolytic system during the screening of vasoactive nonenzymatic drugs.

Exercise, sudden death, electroshock, and emotional disturbances have also been shown to stimulate the human fibrinolytic mechanism.[10] Inasmuch as their common denominator appeared to be stress, a number of neurologic procedures (pneumoencephalography, cerebral

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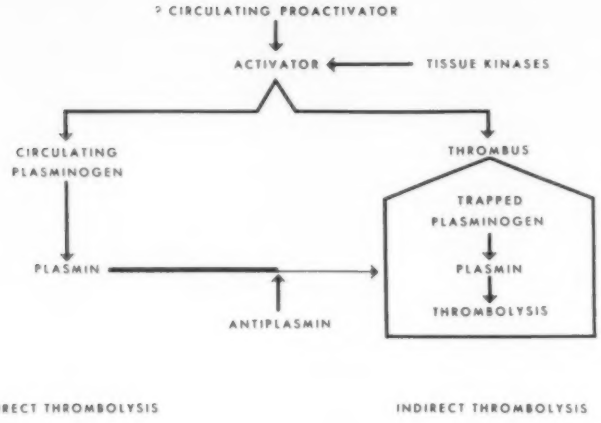

Fig. 1. Suggested mechanisms of thrombolysis

arteriography, myelography, and caloric tests) were chosen for study because they represented various degrees of stress that directly involved the nervous system. In this way, the relation of a neurogenic mechanism to the spontaneous activation of fibrinolysis might be elucidated.

It is useful at this point to discuss the suggested pathways for the spontaneous human thrombolytic mechanism (Fig. 1). The essential step appears to be the activation of plasminogen to form the active enzyme plasmin which is capable of hydrolyzing the internal peptide bonds of the fibrin of clots. The exact mechanism whereby plasminogen is spontaneously activated is not clear, and there is controversy regarding the existence of a separate substance in circulating blood which functions as an activator of plasminogen. Activators or kinases are present, however, in many body tissues and it seems likely that, under appropriate circumstances, they can be released to the circulation where two possible pathways of action appear to exist. The most likely one for spontaneous thrombolysis is that in which the activator is adsorbed onto the thrombus and subsequently activates the plasma plasminogen incorporated within the clot to form plasmin, which then attacks the thrombus from within. Alternatively, plasminogen may be activated in the circulation and the plasmin so formed may directly attack the clot from without. These pathways may, in fact, both be operative, though one objection to the effectiveness of circulating plasmin must be noted. Under ordinary circumstances, the concentration of circulating antiplasmin is such that it probably quickly neutralizes most or all of the circulating plasmin.[11] There is also evidence that plasmin is most effective in breaking down fibrin in the depths of the thrombus protected from the action of antiplasmin.[12] Whether an additional or coincidental step of fibrin denaturation occurs is not yet known.[13] Injected streptokinase is believed to react with a circulating proactivator to form an activator of plasminogen, for it is known that streptokinase alone cannot convert plasminogen to plasmin.

Suggestive evidence that the local release of an activator or proactivator of plasminogen from the vascular wall is responsible for the fibrinolytic activity within vessels is derived from several sources. Mole[14] found an approximately inverse relationship between fibrinolytic activity and the diameter of blood vessels in individuals who had died suddenly. He made the suggestion that such activity might take origin from the vascular endothelium, particularly of the smaller vessels. Todd[15] reported that all tissues except the liver showed scattered foci of fibrinolytic activity consistently related to veins or venous sinusoids, and, to a lesser extent, arteries, except in the lung where they were predominantly related to pulmonary arteries. The well-known phenomenon of recanalization of venous thrombi by thrombus

dissolution in contrast to reorganization may be related to this finding. Astrup and associates[16] demonstrated that the adventitia of the human aorta contained significant amounts of a plasminogen activator, while the intima and media usually were inactive. Kwaan and coworkers[2,17] found that, in addition to vessel wall ischemia, the intravenous or paravenous injection of Adrenalin, serotonin, or acetylcholine not only caused local induction of fibrinolytic activity but a reflex production of lytic activity in veins of the same and opposite arms as well. Atropine was found to block the efferent portion of the reflex arc but not the afferent side. Exercise of ischemic muscles prevented the induction of fibrinolytic activity, but this inhibitory effect was blocked by Prostigmin.[18] These authors concluded that a cholinergic effector mechanism existed in venous, and probably arterial, walls which induced secretion of an activator of plasminogen and that ischemia of the vessel wall due to constriction of the vasa vasorum, produced either by drugs or compression, was the probable stimulus to this mechanism. They also felt that the serotonin released by disintegrating platelets in and around a thrombus might be an additional in vivo stimulus to the cholinergic effector mechanism.[19]

The induction of an increase of fibrinolytic activity in human beings by Pitressin and certain stressful neurologic procedures will now be reported, and the role of the nervous system in fibrinolysis will be assessed. Animals are, unfortunately, not suitable for this type of research inasmuch as their fibrinolytic systems are either not responsible to stimulation or else react only to stimuli of unusually great severity, such as circulatory shock.

MATERIALS AND METHODS

Subjects. All investigations were carried out on either adult medical students or patient volunteers, almost all of whom were males. When a substance was injected, the subject was unaware of the nature of the preparation used or of anticipated side effects.

Fibrinolysis determinations. Lytic activity was determined by the euglobulin lysis time method of von Kaulla and Schultz.[20] The blood samples were kept in ice water and the tests were performed within thirty minutes after drawing the specimens. The euglobulin fraction of plasma is precipitated in this method. This fraction contains all the components of the fibrinolytic system except antiplasmin plus about 25% of the fibrinogen and other clotting factors. After redissolution and clotting with human thrombin, the time of clot lysis can be accurately observed. The sensitivity of this method is very great.

Pitressin injections. Pitressin (20 units) was diluted in 2 cc. of normal saline, following which 1 to 2.5 units were placed in a tuberculin syringe. The intravenous injections into an antecubital vein took less than thirty seconds to administer.

Neurologic procedures. *Pneumoencephalography* was performed on conscious patients, usually premedicated with codeine and Nembutal, using 1 to 2 cc. of 1% procaine for local anesthesia of the lumbar puncture and room air as the instilled gas.

Ventriculography was performed on a conscious patient premedicated with codeine and Phenergan, and 5 cc. of 1% Xylocaine was used for local anesthesia of the scalp.

Cerebral arteriography was performed on conscious patients, usually premedicated with codeine and Phenergan. Percutaneous puncture of either the carotid or vertebral artery in the neck was made, using 5 to 10 cc. of 1% Xylocaine for local anesthesia and varying amounts of 50% Hypaque as the contrast material.

Caloric tests were performed by irrigating the auditory canals for one minute with 50 cc. of ice water.

Lumbar myelography was performed with 6 cc. of Pantopaque on a conscious patient premedicated with codeine and Nembutal.

It should be noted that, in the dosages used, the local anesthetics do not themselves cause any change in fibrinolytic activity. The premedications listed did not induce any fibrinolytic activity, for control lysis times before and after medication were essentially unchanged.

RESULTS

Intravenous Pitressin. *With no circulatory occlusion.* Each of 5 subjects had 1 unit of Pitressin injected into an antecubital vein, while 2 subjects received 2 units. Preinjection control and postinjection serial blood speci-

TABLE 1

INDUCTION OF FIBRINOLYTIC ACTIVITY BY INTRAVENOUS PITRESSIN

Subject	Units of Pitressin	Control lysis time (minutes)	Blood samples* after injection			
			4 minutes	8 minutes	16 minutes	32 minutes
F.K.	1	65	42	32	—	55
J.M.	1	108	38	38	47	72
D.M.	1	90	60	38	30	37
R.M.	1	126	70	62	60	83
D.E.	1	180	44	38	63	90
J.M.	2	116	45	40	60	—
B.S.	2	180	63	62	66	—
Averages		124	52	44		

*Euglobulin lysis times in minutes

mens were drawn from veins in the opposite arm. The average control value for the euglobulin lysis time was one hundred and twenty-four minutes, while the average values for specimens drawn four to eight minutes after injection were fifty-two and forty-four minutes, respectively (Table 1). By thirty-six minutes after injection, most values were rising toward the control level.

With unilateral circulatory occlusion. Nine subjects participated in 11 experiments carried out in the following manner. After a control specimen was taken from an antecubital vein in one or both arms, a blood pressure cuff placed above the antecubital fossa of one arm was pumped to a level 50 mm. of Hg above the individual's systolic blood pressure. Pitressin, 1 to 2.5 units, was then injected intravenously into the opposite arm, and, at regular intervals, blood samples were withdrawn from antecubital veins in both arms. Usually after twelve minutes (range six to seventeen minutes), the cuff was released and sampling of blood, usually bilateral, was continued. In 2 of the experiments, the Pitressin was allowed to circulate for thirty seconds before pumping up the cuff.

One subject was a complete nonreactor to the Pitressin stimulation. Ten of the experiments resulted in the induction of fibrinolytic activity in the unoccluded arm, with the shortest lysis times usually appearing in the four- to eight-minute specimens (Table 2). In addition, in 6 subjects, a decrease in lysis time was noted in the occluded arm prior to the release of the cuff. On 3 occasions, an initial prolongation of lysis time was noted in the two-minute values in the occluded arm, with a marked shortening of lysis time thereafter.

Side reactions. The majority of the subjects experienced side effects, such as pallor, slight nausea, mild abdominal cramps, and a desire to void, shortly after the Pitressin injections. These usually disappeared in four to five minutes, and the intensity of these reactions was not well correlated with fall in euglobulin lysis time. The blood pressure usually rose slightly during these experiments.

Atropine. The intravenous injection of 0.6 mg. of atropine sulfate five minutes before the intravenous injection of 1 unit of Pitressin failed to prevent a marked decrease in lysis time noted four minutes after the latter drug.

Air encephalography. *Pneumoencephalography* was performed on 7 patients. Of these, 3 responded with a marked and prolonged shortening of their euglobulin lysis times (Table 3). The average control value in this group was one hundred and six minutes, while the average value five minutes after the instillation of 20 to 60 cc. of air was twenty-five minutes. Of these patients, 2 maintained their lysis times near this low level for ninety and one hundred and forty minutes, respectively, while 1 of them showed a significant decrease in lysis time in an arm cuffed for ten minutes at a level

50 mm. of Hg above systolic blood pressure. A fourth patient fainted before the instillation of air, and her fibrinolytic activity will be reported below.

During pneumoencephalography, 3 patients failed to show any shortening of lysis time despite systemic reactions indistinguishable from those in individuals whose fibrinolytic system

TABLE 2

INDUCTION OF FIBRINOLYTIC ACTIVITY BY PITRESSIN IN OCCLUDED AND NONOCCLUDED ARMS

Subject	*Units of Pitressin*	*Blood samples* after injection*								
		0 to 2 minutes		*4 minutes*		*8 to 12 minutes*		*16 to 18 minutes*		*24 to 40 minutes*
W	2	180		60		40	//	41		73
		180		—		**97**	//	**59**		—
K	1	72		29		—	//	31		90
		110		—		**51**	//	—		—
B†	1	119		60		92	//	73		92
		120		**126**		**48**	//	**100**		**80**
D	1	160		100	//	108		180		180
		—		**360**	//	**95**		—		—
N†	2	83		37		38	//	58		85
		83		**60**		**58**	//	**60**		**66**
L	2	180		50		61	//	66		180
		180		**68**		**50**	//	**105**		—
R	2.5	150		35		40		35	//	56
		115		**115**		—		**95**	//	**65**
R	2	116		41		40	//	55		87
		90		**150**		**106**	//	**62**		—
Wh	2	180		180		112	//	165		—
		180		**180**		**180**	//	—		—
Bu	2	150		83		97	//	104		145
		170		**180**		**180**	//	**114**		—

*Euglobulin lysis times in minutes
† Pitressin circulated for 30 seconds before cuff application
// Cuff release
Light face = nonoccluded arm
Bold face = occluded arm

TABLE 3

INDUCTION OF FIBRINOLYTIC ACTIVITY BY PNEUMOENCEPHALOGRAPHY

Subject	*Control lysis time (minutes)*	*Blood samples* after air injection*			
		5 minutes	*35 minutes*	*90 minutes*	*140 minutes*
B	106	31	27	—	—
H	92	25	—	—	31
Le	120	20	—	35	—
	120	**35**	—	—	—
Average	106	25			

* Euglobulin lysis times in minutes
Light face = nonoccluded arm
Bold face = occluded arm

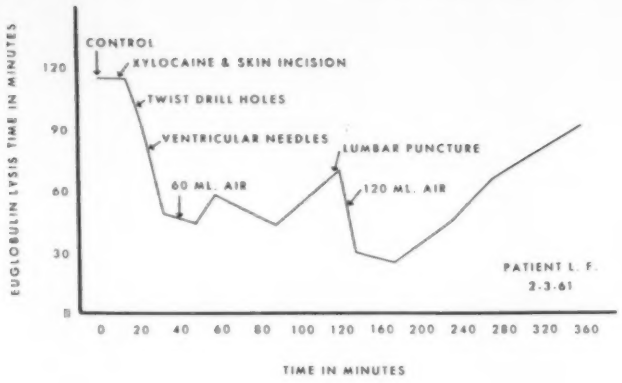

Fig. 2. Induction of fibrinolytic activity by ventriculogram plus pneumoencephalogram

was markedly activated. In 1 of these patients, the air failed to enter the ventricular system.

Ventriculography. One additional patient had a ventriculogram made, which was followed immediately by a pneumoencephalogram. There was marked decrease in lysis time from a control value of one hundred and ten to forty-nine minutes just after twist drill holes were made in the skull and ventricular needles were placed in both ventricles. Marked pallor and sweating occurred during the latter procedure. The injection of 60 cc. of air further lowered the lysis time to forty-four minutes, and it then rose to sixty-nine minutes in the next hour and a quarter. Almost complete replacement of the cerebrospinal fluid by air for the following pneumoencephalogram caused severe headache, sweating, and vomiting. The lysis time was quickly lowered to twenty-five minutes, from which point it slowly rose (Fig. 2).

TABLE 4

INDUCTION OF FIBRINOLYTIC ACTIVITY BY CEREBRAL ARTERIOGRAPHY

	*Blood samples**	
Subject	*Control*	*5 to 15 minutes after dye injection*
G.G.	108	72
R.S.	105	60
J.G.	114	105
C.S.	112	110
D.B.	110	26
L.F.	120	74

*Euglobulin lysis times in minutes

Cerebral arteriography. Studies were made of 5 patients during carotid arteriography and of 1 during vertebral arteriography. Of these, 2 showed moderate shortening of the lysis times five to fifteen minutes after injection of the dye and 2 patients showed no response (Table 4). In 2 other patients (D.B. and L.F.), the major portion of the decrease in lysis time occurred just after the arterial puncture and prior to the injection of dye (Fig. 3). The dye appeared to cause an additional small decrease.

Caloric tests. During these tests, 4 subjects were studied. One arm was occluded at a level 50 mm. of Hg above systolic blood pressure just before the irrigation with ice water. Each subject showed only a mild shortening of lysis time two to four minutes after irrigation, and, in 3 instances, an immediate rebound to values higher than the control was noted. Only 1 subject showed a slight shortening of lysis time in the occluded arm.

Myelogram. A lumbar myelogram in 1 patient failed to cause any significant change in the euglobulin lysis time.

Fainting. One patient with a control lysis time of ninety minutes fainted just after a lumbar puncture was performed for a pneumoencephalogram. Ten minutes after the faint, the lysis time was forty-two minutes, and, twenty minutes after the faint, it had decreased to seventeen minutes. At this point, the air

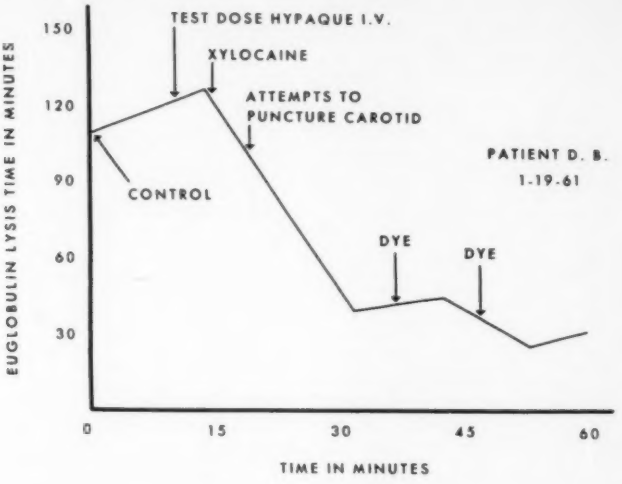

Fig. 3. Induction of fibrinolytic activity by cerebral arteriography

injection began and, one hundred and ten minutes later, the euglobulin lysis time was only forty minutes (Fig. 4).

Controls. *Circulatory occlusion.* To test the completeness of circulatory occlusion at a level 50 mm. of Hg above systolic blood pressure, such an occlusion was made in one arm just prior to the injection of 1 cc. of a standard dilution of radioactive iodinated serum albumin (RISA) into the opposite arm during a blood volume determination. Samples were drawn from both arms at five- and ten-minute intervals. No increase over background radioactivity was found in counts of the samples from the occluded arm, while counts of blood from the opposite arm showed a significant increase in radioactivity.

Injection of inactive substance. Using 5 subjects, in 4 of whom shortened lysis times were known to develop in response to Pitressin, the cuffed arm experiments with unilateral circulatory occlusion were repeated, except that 0.5 to 1.5 cc. of normal saline was substituted for the Pitressin. No shortening of lysis time was noted in either arm, and the average results in 3 subjects are shown in Table 5. The other 2 subjects had lysis times of over four and five hours, respectively.

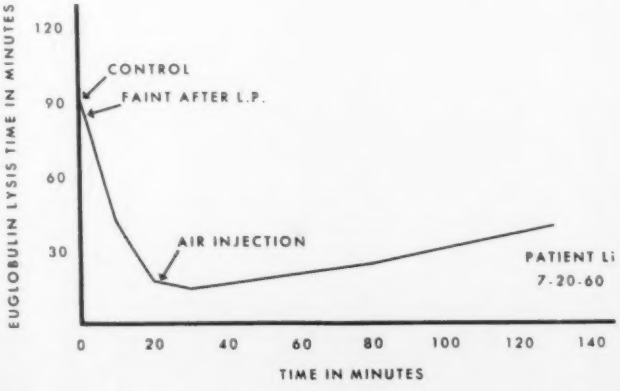

Fig. 4. Induction of fibrinolytic activity by a faint plus pneumoencephalogram

TABLE 5

INDUCTION OF FIBRINOLYTIC ACTIVITY BY SALINE IN OCCLUDED AND NONOCCLUDED ARMS
(Average values in 3 subjects)

	Control lysis time (minutes)	*Blood samples* after injection*		
		4 minutes	*8 minutes*	*16 minutes*
Nonoccluded	93	126	102	93
Occluded	94	99	120	106

*Euglobulin lysis times in minutes

DISCUSSION

Intravenous Pitressin appears to be a fairly consistent activator of the human fibrinolytic system, for, in 17 of 18 subjects, the euglobulin lysis time was significantly decreased from control values; that is, the clotted euglobulin fraction redissolved in a markedly shorter time than before injection.

The control studies established that a circulatory occlusion at a level of 50 mm. of Hg above systolic blood pressure did not induce any significant increase in fibrinolytic activity below the cuff or in the opposite arm and that an occlusion at that level prevented the passage of RISA and, by inference, any circulating hormonal factors. A decrease in euglobulin lysis time in veins below the cuff was noted in 6 of 11 subjects given Pitressin, 1 of 3 patients having pneumoencephalograms, and in 1 of 4 subjects having caloric tests. It appears, then, that the induced fibrinolytic activity below the cuff came about by virtue of some reflex mechanism which caused the release of some agent or agents from the vessel wall. It is possible that this neurogenic mechanism is invoked by any endogenous activator of fibrinolysis, whether a drug or a nonspecific stressful procedure.

In 2 experiments, the Pitressin circulated freely for thirty seconds prior to the occlusion. It might be argued that the drug itself trapped in veins below the cuff, though much diluted, might have caused a local fibrinolytic activation. That this was not the case is suggested by the results of a previous observation that Pitressin placed within an occluded vein for five minutes failed to induce any fibrinolytic activity within that vein. This also agrees with the observation of Kwaan and associates[2] that the local induction of fibrinolytic activity by drugs is suppressed during a period of active ischemia. A second possibility is that during the period of free circulation, some lysis-inducing factor was brought to, or produced in, the extremity and then trapped there when the circulation was occluded. If this were so, then the results in both arms, particularly in the early specimens, should have been in agreement. Actually, at the same time intervals, they were quite dissimilar. It appears that a neurogenic mechanism was probably operative in these 2 experiments as well as in the others.

As stated previously, Kwaan and his colleagues[21] feel that ischemia of the vessel wall, whether induced by compressed or by drug administration, is the probable stimulus to a cholinergic effector mechanism residing in vessel walls. Pitressin, which is known to cause smooth muscle contraction, could conceivably act locally to constrict the vasa vasorum and thus stimulate this effector mechanism and possibly cause afferent stimulation as well. However, it seems likely that a stimulus other than, or in addition to, ischemia of the vessel wall can activate the effector pathway and explain the effect of Pitressin, for, in our control experiments, there was no induction of fibrinolytic activity in blood held in the veins of ischemic limbs for twelve minutes. The possibility that the stimulus is neurogenic and acts centrally appears worthy of consideration.

A fairly well-established cholinergic effector mechanism subserves active vasodilation, and it may also function in endogenous production of fibrinolysis. The major component of the vein wall is the adventitia, in and around which there is an extensive ramification of afferent and efferent autonomic nerve fibers.[22] The latter are composed of both vasocon-

strictor and vasodilator fibers. The vasodilator transmitter substance in arterial walls, and presumably in vein walls, is generally accepted to be acetylcholine, for it is blocked by atropine and potentiated by physostigmine. Venomotor regulation is thought to be part of general vasomotor regulation under the control of reflexes similar to those affecting the arterial tree.[23] Though a plasminogen activator has not yet been isolated from vein adventitia, it seems reasonable to assume that it might reside in that layer as it does in the aorta, and release might be accomplished by reflex activation of the cholinergic fibers just described. Diffusion through the thin endothelial lining of the vein into the circulation might then occur. One objection to this hypothesis is that it endows the released acetylcholine with two activities—vasodilation and the release of a plasminogen activator. However, a precedent for the possession of two activities by a single molecule is found in synthetic vasopressin which has both smooth muscle stimulating and antidiuretic actions. If acetylcholine plays a role in the induction of fibrinolysis, it should be possible to inject this substance and thereby induce a lytic tendency. In human beings, it is necessary to inject enough acetylcholine systemically to cause a brief period of shock before a fibrinolytic tendency is induced.[24] Small amounts of injected and freely circulating acetylcholine are without effect. Kwaan and associates[2] have shown, however, that injection of acetylcholine into or near an occluded vein is effective in inducing a lytic tendency, and the action of this substance may, therefore, be a function of its local concentration.

The central efferent pathway for the cholinergic vasodilator mechanism appears to be quite extensive.[23] In dogs and cats, fibers have been found from frontal, temporal, and motor areas of the cerebral cortex which pass down the internal capsule to the hypothalamus where they may synapse or pass to the mesencephalon. Both the hypothalamus and mesencephalon act as major integrating areas for pressor and depressor activity of the vasodilator system. Fibers that synapse in or pass through the mesencephalon reach sympathetic cells in the lateral horns of the spinal cord. In addition, cells from pressor and depressor areas in the medulla send axons to this region of the cord. Thus, there is much opportunity for afferent impulses originating either peripherally or centrally to affect this cholinergic system. Pitressin and other drug stimulators of fibrinolysis might act by being brought via the blood stream to these central areas where they could initiate impulses in the efferent system.

The redistribution of blood in emergency situations and in exercise and emotional states may occur via this cholinergic system through the integrative action of the hypothalamus. These stressful physical and emotional situations are the same as those in which spontaneous increases in fibrinolysis have been found, and this similarity suggests that the same cholinergic effector mechanism responsible for vasodilation may be responsible for the endogenous production of a lytic tendency.

Our failure to inhibit the induction of fibrinolytic activity, using 0.6 mg. of atropine before Pitressin stimulation, and Sherry and associates' failure to do so, using 3 to 4 mg. of atropine before electroconvulsive therapy,[25] may be due to the fact that these two strong stimulators of fibrinolysis overwhelmed the small amounts of atropine used systemically. Local use of atropine might show inhibition, and we are now studying this possibility.

Pneumoencephalography in 3 of 7 patients produced the strongest and longest lasting lysis thus far encountered. It appears to work merely as a nonspecific stressful stimulus of great intensity, probably by virtue of the pain, apprehension, and hypotension induced by the procedure. Excitation at many central levels of a neurogenic activating mechanism for fibrinolysis could thus occur. Of great interest was the marked shortening of lysis time in the patient who fainted before the air was injected and in the patient who became pale and sweaty during the placement of the ventricular needles. Barcroft and associates[26] showed that, in a faint, the amount of human forearm blood was increased and that this was presumably due to sympathetically mediated active vasodilation. These data further suggest that the cholinergic mechanism active in producing vasodilation may also cause release of a plasminogen activator. Noble and Taylor[27] found that the urine of human subjects

who fainted contained an antidiuretic substance believed to be posterior pituitary hormone. They postulated that reflex stimulation of the neurohypophysis during a faint caused the excretion of vasopressin. They noted that other procedures found to cause stimulation of the neurohypophysis in human beings were electroshock and ischemic muscle pain, while, in dogs, exercise, emotional stress, and the administration of acetylcholine also caused the liberation of vasopressin. As mentioned previously, these same stimuli cause an increased fibrinolytic tendency, and perhaps released vasopressin acts as an additional afferent stimulus to the neurogenic efferent mechanism just discussed.

Whether a release of Adrenalin by air encephalography and other stressful neurologic procedures is responsible for the increased lysis tendency is debatable. It is true that the adrenal medulla is stimulated under these circumstances, but it has been shown that at the rate Adrenalin is released on nerve stimulation, its general vascular effects are slight.[28] It is thought that vasomotor control is dominated by the neural component and that the main function of the released Adrenalin is to increase metabolic processes in vital organs. On the other hand, the intravenous administration of epinephrine and other pressor amines has been found to promptly produce a significant activation of fibrinolysis.[29] Small amounts of Adrenalin have been shown to produce vasodilation in skeletal muscles, but this is believed to be an indirect effect due to the glycogenolytic action of the drug which causes release of lactic acid.[28] Vasodilation alone does not induce fibrinolytic activity.[30] Perhaps the vasoconstricting effect of administered Adrenalin provokes a counter reaction in the cholinergic vasodilating system which, while not strong enough to overcome the vascular effect of the drug, is sufficient to stimulate those fibers affecting the release of a plasminogen activator.

The induction of fibrinolytic activity by arteriography was less than that noted following pneumoencephalography and may be related to the less stressful nature of the former procedure. The fact that, on two occasions, significant enhancement of fibrinolysis was obtained merely from needle puncture of the carotid artery makes it necessary to interpret with caution the results of intracarotid instillation of enzyme-containing fibrinolytic preparations. Clot lysis might well be due to the induction of a fibrinolytic state by the arterial puncture alone and have little to do with the infused preparation. This is also true of the situations wherein the infusions were given intravenously but in which pretreatment arteriograms were made. The infused fibrinolytic enzyme solutions might also produce fever and chills which in themselves cause a very marked fibrinolytic reaction.[31]

The results of the caloric tests show the rather small fibrinolytic effect of a mild and shortlived stress. It is not surprising that myelography did not induce a lytic tendency, for it is, relatively speaking, an even milder stress.

It is not known why some people react poorly or not at all to stimuli that induce fibrinolytic activity in others. Hence, we have no explanation for our nonreactors who, so far as we know, had not had a recent activation of their fibrinolytic systems that might have put them into partial refractory states.[31] We also cannot yet explain why a few individuals had a prolongation of their lysis times at the onset of the experiment before a marked shortening developed.

To sum up the neurogenic hypothesis of human fibrinolytic activation, it appears possible that many types of peripherally and centrally induced afferent stimuli may act within the central nervous system to excite an efferent mechanism, presumably similar to the cholinergic mechanism which subserves vasodilation, to release an activator of plasminogen from vascular walls. Further work to clarify this hypothesis, as well as studies of nonenzymatic drug stimulation of fibrinolysis, appears worthwhile. If a simple drug could be found which would endogenously and repetitively stimulate the body's own fibrinolytic system by causing the release of a plasminogen activator without inducing serious side effects, thrombolytic therapy of cerebrovascular disease would be significantly advanced.

SUMMARY

The effects of Pitressin and certain stressful neurologic procedures on the human fibrinolytic system have been studied, and the role of

the nervous system in fibrinolysis has been discussed.

In 17 of 18 volunteers, intravenous Pitressin rapidly induced a 38 to 80% reduction in euglobulin lysis time. On 6 of 11 occasions in which the circulation of one arm was occluded prior to the injection of Pitressin into the other arm, a reduction of lysis time was noted in both arms. Pneumoencephalography in 3 of 6 patients produced the strongest and longest lasting fibrinolytic activity thus far encountered. Of these 3 patients, 1 also had reflex-induced lytic activity appear in a cuffed arm. Marked shortening of lysis time was noted in 1 patient who fainted before a pneumoencephalogram and in another who became pale and sweaty during insertion of ventricular needles. Cerebral arteriography in 2 of 4 patients induced a moderate increase in fibrinolytic activity and, in an additional 2 patients, the needle puncture alone caused the same result. Caloric tests in 4 patients caused a brief, mild lysis, and, in 1 of these, reflex-induced fibrinolytic activity also appeared. A myelogram was without effect, and 0.6 mg. of atropine given intravenously failed to prevent the enhancement of fibrinolysis by Pitressin.

It appears possible that many types of peripherally and centrally induced afferent stimuli may act within the central nervous system to excite an efferent mechanism, presumably similar to the cholinergic mechanism which subserves vasodilation, to release an activator of plasminogen from vascular walls.

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Morphologic findings in studies of regeneration of synapses in the mammalian spinal cord

C. C. Turbes, D.V.M., and L. W. Freeman, M.D.

EARLIER WORK concerning regeneration of synaptic connections has been studied primarily in amphibia.[1–8] Sperry and Miner[7] and Stevens[8] showed that functional regeneration of synapses followed sectioning and anastomosis of the central process of the dorsal root of one side to the proximal stump of the dorsal root of the opposite side. A number of studies[9–13] have shown that the dorsal roots and dorsal columns of mammals have adequate regenerative capacities. There is no functional or morphologic evidence for reestablishment of synaptic connections reported in the mammalian studies.

A number of authors[11–13] have reported that regenerating dorsal root fibers are confronted with a barrier at the neurilemmal-glial junction.

Previous studies[14,15] have shown that peripheral nerve fibers inserted into the spinal cord grow profusely. Theorizing that regenerating dorsal root fibers would grow and reestablish synaptic connections more readily, it was decided to insert the neurilemmal portion of the dorsal roots into the spinal cord to the depths of the gray columns.

METHODS

Under pentobarbital sodium anesthesia and aseptic conditions, 27 female mongrel dogs weighing 8 to 10 kg. were subjected to laminectomy of two vertebrae. Usually the spine and laminae were removed. This exposed the spinal cord at the lumbar enlargement. The epidural adipose tissue was removed by gentle suction. The dura was then stabilized by two sutures and carefully opened to the proper length with the assistance of a grooved director. The incised dura was further retracted with sutures, and these segments of the spinal cord were exposed as completely as possible.

Two different methods were used in binding the rootlets to aid in insertion. In 14 animals, a 5-0 silk suture was passed around a dorsal root where the rootlets become confluent peripherally near the ganglion. This ligature was not tightened but was used to keep the rootlets in a compact bundle. The component rootlets were then carefully avulsed or sectioned at their attachment to the spinal cord. The binding suture was then moved toward the free ends of the rootlets and tied just tightly enough to hold them together. The pia above the dentate ligament over the lateral funiculus was incised and an opening was made in the lateral funiculus by directing the point of a No. 11 Bard-Parker scalpel blade toward the ventral gray column. The bundle of rootlets was guided into the depths of the incision with a curved eye forceps (Figs. 1 and 2). Gelfoam was compressed against the inserted dorsal root until extravasated blood at the cord incision clotted, holding the root in place within the spinal cord. In 13 later operations, the avulsed rootlets were clotted together with blood and then guided into the incision in the spinal cord. At times, some of the rootlets broke away from the clot during their insertion into the cord. These rootlets were inserted into

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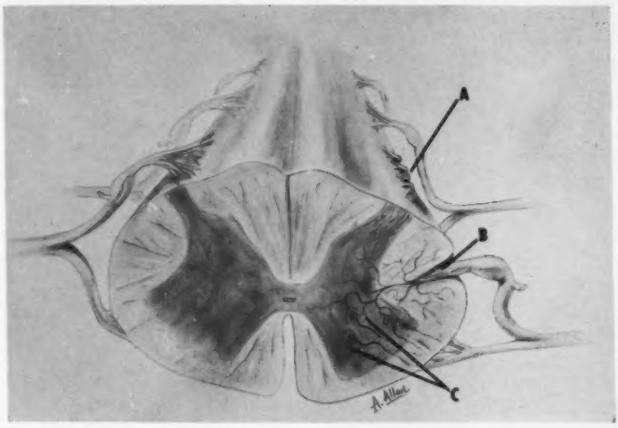

Fig. 1. Diagram of spinal cord showing dorsal root inserted into lateral funiculus. [A] Cut end of dorsal root. [B] Dorsal root inserted into lateral funiculus. [C] Pattern of root regeneration around ventral horn neurons

the cord adjacent to the already indwelling rootlets or into a new incision nearby. The dura and other tissue layers were then closed, terminating the operative procedure.

The same surgical approaches were used to operate upon 12, 1-month-old calves. The avulsed or cut dorsal rootlets were clotted together in each case and inserted into an incision in the spinal cord. The surgical procedures were accomplished with greater ease in the calves because of the greater size of structures involved.

The control animals used consisted of 5 dogs and 3 calves. The surgical approaches were the same as those used in the experimental animals, except for inserting the dorsal root. The dorsal roots were doubly ligated near the ganglion. The portion of the root between the ligature and its entry into the spinal cord was removed. An incision was made in the spinal cord; no nerve root was inserted. All controls were kept for one and one-half years.

To determine reestablishment of synaptic connections, stimulation of the regenerating

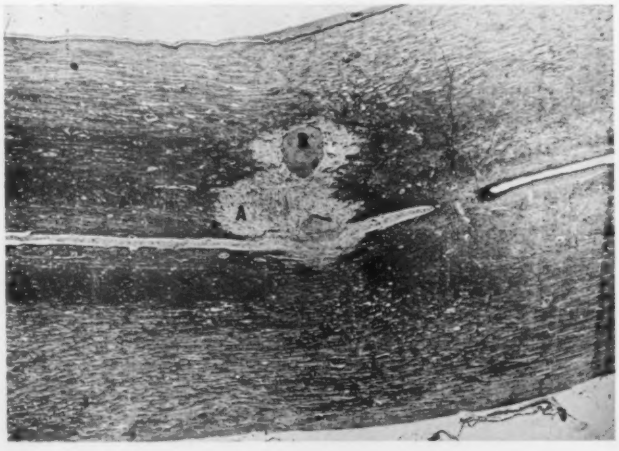

Fig. 2. Dorsal root fibers within spinal cord of the calf. [A] Region of dorsal root fiber regeneration. [B] Dorsal root fiber. Bodian stain. × 800

dorsal root and observations for muscle contraction were made. All stimulation procedures were terminal and were performed in animals kept to the end of the experimental period of one and one-half years. The only animals stimulated were those in which no problems were apparent during surgical insertion of the dorsal root into the lateral funiculus. Some animals of this group were also removed because of extensive scar tissue encountered during the dissection preparatory to stimulation. In all, 12 dogs and 9 calves were stimulated. The parameters of electrical stimuli were determined on normal. A Grass model S-4 stimulator was used.

All experimental and control animals were sacrificed by using an overdose of sodium pentobarbital. The block of vertebral column containing the spinal cord and the regenerating dorsal root was placed in excessive volumes of neutral 10% formalin. On complete fixation, the spinal cord and root were carefully dissected, imbedded in paraffin, and serially sectioned. Hematoxylin and eosin, Mallory triple, and buffered thionine were stains routinely used. Several special nerve fiber stains were used: Cajal, Bodian, Bielschowsky, DeMeyer modification of Hortega, and Rasmussen silver impregnation of synaptic endings. The same regions of the spinal cord in 3 normal calves and 3 dogs were treated in the same manner and the same staining procedures were used.

RESULTS

In both experimental and control dogs, the following general pattern of symptoms was observed after surgery.

1. Consistently, there was a defective placing reaction of the rear extremity on the operated side. This symptom was most severe during the first three days and gradually disappeared in the second week. In some animals, slight defects were observed while climbing stairs until the end of the experimental period.

2. An area of anesthesia and hypesthesia on the operated side extended from the dorsal midline to the patella anteriorly and posteriorly to the level of the tuber calcanei. It was difficult to demonstrate this deficit after the first postoperative week.

3. The tail was usually deviated toward the unoperated side. On wagging the tail, the operated side showed paralysis. These deficits were not apparent after the second week.

4. Fasciculation of muscles of the operated rear limb was evident between the fourth and sixth postoperative weeks. The muscles that most frequently showed fasciculation were the semitendinosus, semimembranosus, and vastus lateralis.

The symptoms observed in the experimental and control calves were similar to those seen in the dogs. Fasciculation of the muscles of the operated rear limb was not observed. As in dogs, minor placing defects of the operated limb persisted for the experimental period.

The results of stimulation of the regenerating dorsal roots were encouraging but incomplete. Muscle contraction was elicited on stimulation of the operated dorsal root in 3 calves and 5 dogs of the group tested. More intensive and expanded physiologic studies were undertaken and will be reported later.

Microscopic findings in the dogs and calves are similar and will be considered together.

The findings can best be presented by describing the association of the growing dorsal root fibers. Nerve fibers were found related to the following tissue structures: vascular, neuronal, ependymal, and connective. In this report, more consideration will be given to the neuronal, regenerating root fiber interrelations. These were found to be perisomatic and peridendritic nerve fibers and were associated with synaptic-like structures different from the normal (Figs. 3 and 4). Regenerating root fibers had extended beyond the inserted dorsal roots by the eight month. Neurons with perisomatic and peridendritic regenerating root fibers were closely associated to the inserted dorsal roots (Fig. 5). These neurons were always in fields where growing root fibers, neurilemmal cells, fibroblasts, and connective tissue fibers were located. The neuropil in these regions was partially depleted and replaced with ingrowing tissue elements. Where these elements invaded the area of the central canal, regenerating root fibers were interspersed between ependymal cells.

The synaptic-like structures were never observed with growing root fibers unless they were associated with dendrites or cell bodies of the neurons. Neurons in the same section, but not in the area of dorsal root growth, never

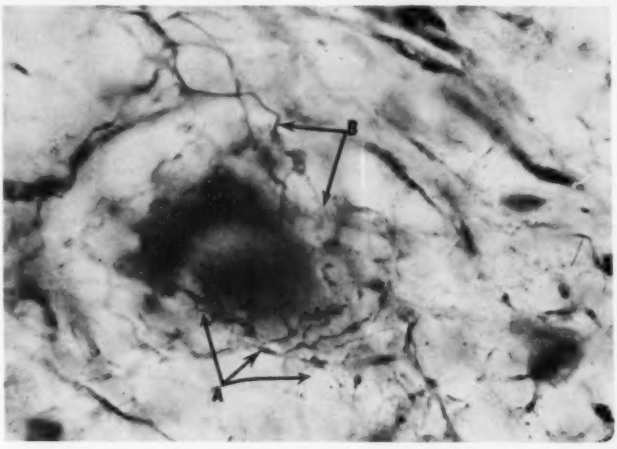

Fig. 3. Neuron in the calf showing regenerating perisomatic nerve fibers and associated synaptic-like endings. [A] Perisomatic nerve fibers with synaptic-like endings. [B] Perisomatic fibers approaching the neuron. Bodian stain. × 800

showed these peridendritic or perisomatic nerve fibers or the regenerating synapses (Fig. 6). The regenerating peridendritic and perisomatic nerve fibers and associated synapses were stained with stains that are not used to demonstrate the terminal axons and synaptic endings in normal spinal cords. By comparison, the same staining procedures—Bodian and modified Cajal—were used on normal dog and calf spinal cords and no synaptic endings could be demonstrated. Also, the same stains were used on sections of the spinal cord of control dogs and calves. No normal or abnormal synaptic endings were found. Special synaptic ending stains were used, and normal synaptic endings are demonstrated (Fig. 4). None of the other findings associated with growing dorsal roots were observed in control spinal cords.

DISCUSSION

This experiment was a preliminary effort to see if synaptic connections would reestablish and if synaptic transmission and correlated

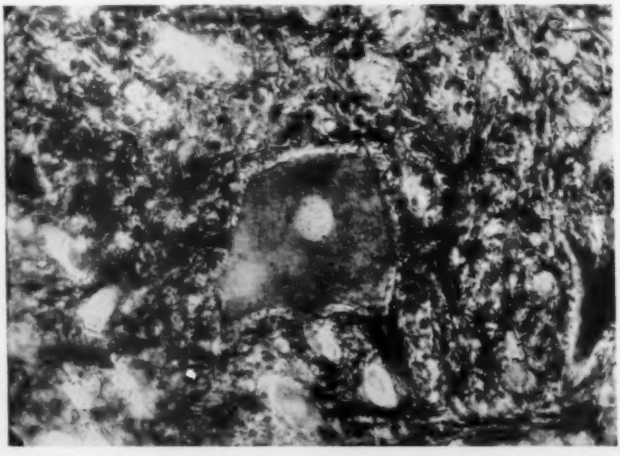

Fig. 4. Neuron in the calf showing normal synaptic endings. Modified Bielschowsky stain. × 600

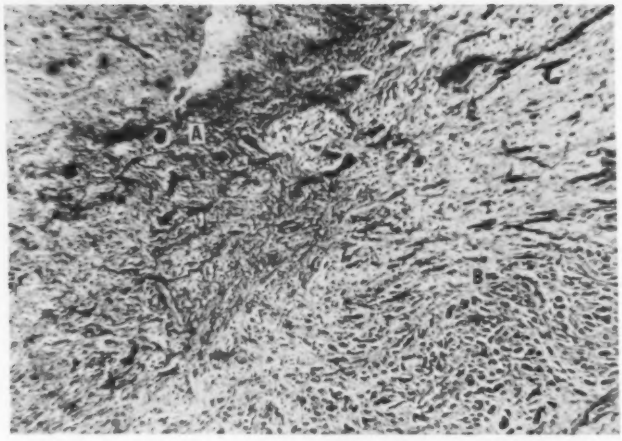

Fig. 5. Group of neurons in the calf in an area of regenerating dorsal root fibers near a dorsal root fiber. [A] Group of neurons adjacent to a root fiber [B]. Bodian stain. × 100

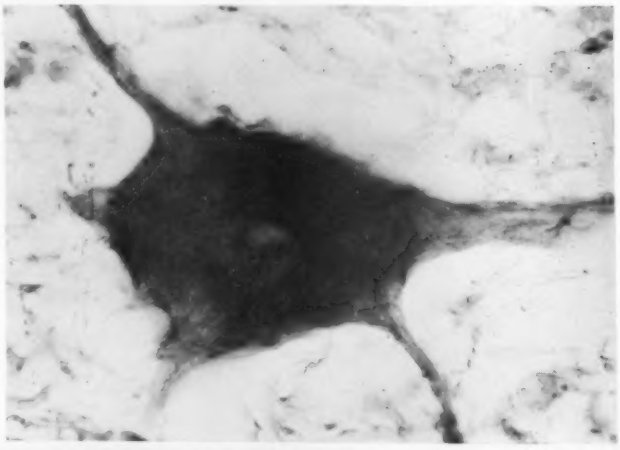

Fig. 6. Neuron in the calf outside of a field containing regenerating dorsal root elements. No regenerating peridendritic or perisomatic nerve fibers or synaptic endings are apparent. Bodian stain. × 800

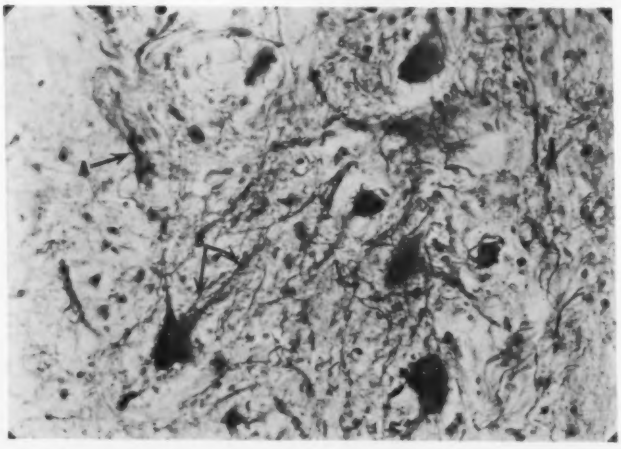

Fig. 7. Group of neurons in the dog associated with growing dorsal root fibers. [A] Regenerating root fibers. [B] Peridendritic nerve fibers. Bodian stain. × 200

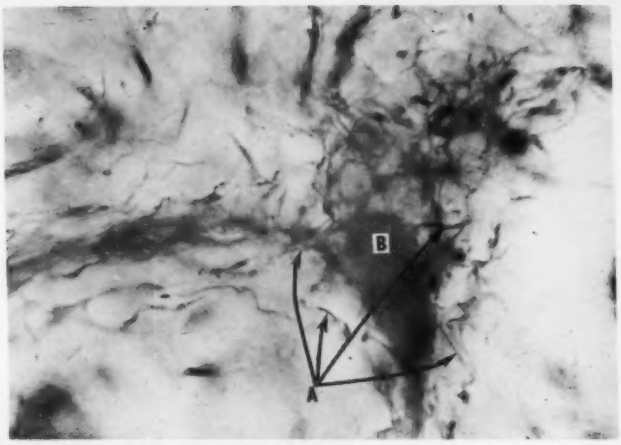

Fig. 8. Neuron in the dog enshrouded with peridendritic and perisomatic nerve fibers. [A] Perisomatic nerve fibers. [B] Neuron cell body. Bodian stain. × 800

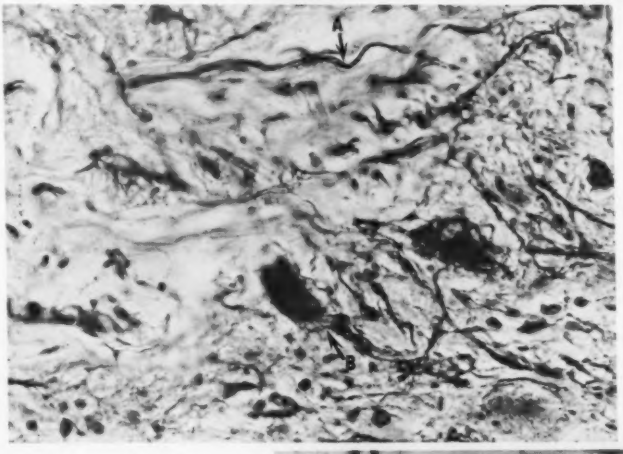

Fig. 9. Neurons in the calf in a field with regenerating dorsal root fibers. [A] A regenerating dorsal root fiber. [B] A synaptic-like ending of perisomatic nerve fiber network. Bodian stain. × 250

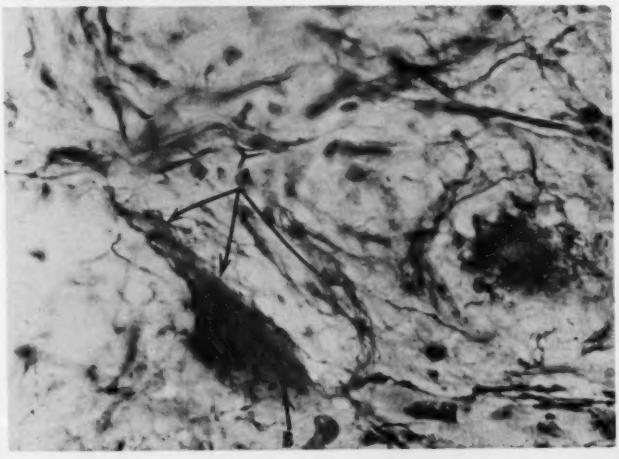

Fig. 10. Two neurons in the calf showing extensive collections of perisomatic and peridendritic nerve fibers and synapses. [A] Perisomatic and peridendritic nerve fibers. [B] Synaptic endings on neuron cell body. Bodian stain. × 800

morphologic findings could be shown experimentally. It was not primarily designed to study the functional recovery that may be associated with regeneration of synapses.

Consistently, in both dogs and calves, the fields containing regenerating nerve fibers, neurilemmal cells, and connective tissue were associated with the inserted dorsal root. As these nerve fibers related to neurons in these areas, they became arranged peridendritically and perisomatically with synaptic endings (Figs. 7, 8, 9, and 10). By comparison, spinal cord sections from control animals had a depleted neuropil and persistent normal terminal nerve fibers and synapses. However, in these cases, special synaptic ending stains[16,17] had to be used to demonstrate the terminal nerve fibers, and still they were not comparable with the regenerating peridendritic and perisomatic nerve fibers and synaptic endings.

The regenerating nerve fibers from the peripheral stump of an intercostal nerve inserted into the spinal cord supplied growing nerve fibers, neurilemmal cells, fibroblasts and connective tissue but no regenerating peridendritic or perisomatic nerve fibers or associated synapses.[14,15]

SUMMARY

The neurilemmal portion of the central stump of a dorsal nerve root was inserted through the lateral funiculus near the ventral horn. Regenerating peridendritic and perisomatic nerve fibers and synaptic endings were found on neurons in regions adjacent to the inserted dorsal root. Regenerating nerve root fibers with neurilemmal cells, fibroblasts, and connective tissue were found growing around blood vessels and between ependymal cells in these areas.

Preliminary physiologic studies indicate reestablishment of functional synaptic connections determined by stimulating the regenerating dorsal root and observing muscle contraction.

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Autotransplantation of anaplastic astrocytoma into the subcutaneous tissue of man

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BECAUSE BRAIN TUMORS in man cannot be easily biopsied, objective evaluation of any form of anticancer therapy is difficult. However, if autotransplants of gliomatous brain tumors in subcutaneous tissue would grow, the effects of various types of anticancer therapy could be determined in a practical manner.

About 11% of extracranial malignant neoplasms metastasize to the brain.[1] However, rarely do brain gliomas metastasize to other parts of the body. Instances are known in which massive infiltration of the scalp tissues has occurred postoperatively, but no evidence of spread to distant organs was found at autopsy.[2] The frequency of primary brain tumors is not low enough to explain the rare metastasis of these neoplasms. Cushing's study[3] of the admissions to the Johns Hopkins Hospital showed primary brain tumors in 40 of 1,000 admissions. Earle[1] reported 99 primary brain tumors in a series of 3,946 consecutive autopsies. Relatively few cases of the spread of brain gliomas to other parts of the body have been reported.[4–8]

In animals, Zimmerman[9] has induced gliomas by chemical means, and, in mice, he has maintained these induced growths as subcutaneous implants for many years. Perese and Moore[10] have induced brain tumors in mice by intracranial implants of different forms of methylcholanthrene and found that these tumors would grow actively in subcutaneous tissue. From their studies, they concluded that such brain tumors in mice resemble morphologically spontaneous brain tumors in man.

In August 1957, we performed several subcutaneous autotransplants of an anaplastic astrocytoma in a patient. One of us (W.H.B.) mentioned this patient at the April 1959 meeting of the Harvey Cushing Society. He also referred to this patient in his report on successful autologous glioma transplants.[11] In a recent article, Grace and associates[12] reported 6 patients with glioblastoma multiforme who had subcutaneous autografts of their tumors, with successful takes in 2 patients. They studied the reactivity of the patients to the tumor or brain tissue antigens. The following is a complete report of our case.

CASE REPORT

P. W., a 47-year-old man, had complained of "sluggishness" for six months and frontal headaches for six weeks before admission to another hospital on March 6, 1957. Examination at that hospital revealed some mental confusion, slight blurring of the optic disks, and some weakness of the left facial muscles. An electroencephalogram showed 4- to 6-per-second slowing on the right side, much more in the frontal and temporal areas. A right cerebral angiogram made March 8 showed a large frontal lesion. At operation on March 12, a soft, brownish, necrotic tumor (histologically, anaplastic astrocytoma) was found in the right frontal lobe. The tumor was partially removed, and postoperatively the patient did well. He

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was discharged from that hospital on March 23, 1957.

On June 10, 1957, the patient returned to work. However, in early July of 1957, he complained of right frontal headaches associated with mental confusion, nausea, and vomiting. On July 25, the patient was admitted for the first time to the Manhattan Veterans Hospital. Examination showed a healed craniotomy scar in the right frontal area, blurred optic disks with some hemorrhages, some apraxia of the left hand, and slight left facial weakness. An electroencephalogram showed diffuse slow voltage activity, most marked in the right frontal and temporal areas. A right cerebral angiogram at this time showed a shift of the anterior cerebral artery to the left. A tumor stain was noted in the right frontal-temporal area. On August 15, the craniotomy was opened and minimal increased pressure was found. The right frontal lobe was involved and was resected with tumor tissue. Histologically, the tumor was composed largely of small dark cells and some large, bizarre cells and resembled an anaplastic astrocytoma (Fig. 1).

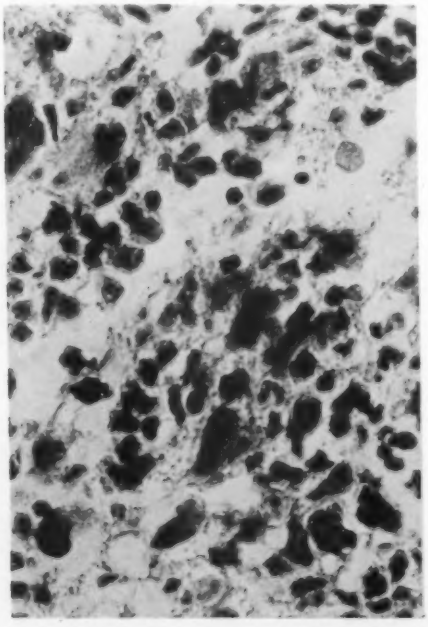

Fig. 1. Brain tumor tissue, operation August 15, 1957. Suspension of this tumor was implanted into subcutaneous tissue of each thigh. Histologically, typical anaplastic astrocytoma. × 992

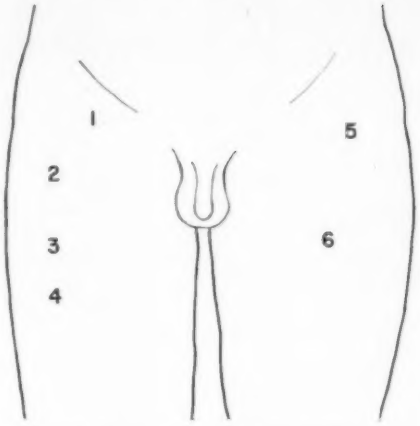

Fig. 2. Sites of tumor tissue implants in subcutaneous tissue of each thigh

At operation, a saline suspension of this tumor tissue was made, and under sterile conditions, with a trocar, 6 implants were placed in the subcutaneous tissue, 4 in the right anterior and 2 in the left anterior thigh (Fig. 2). Postoperatively, the patient's course was uneventful. The first evidence of tumor growth was palpable masses over the right anterior thigh found September 19, 1957, thirty-four days after implantation. The patient was discharged November 4. While at home, he was ambulatory, mentally clear, and self-sufficient. The patient was seen regularly in the clinic, and, on November 22, ninety days after implantation, the No. 3 implant (Fig. 2) was biopsied and showed typical anaplastic astrocytoma tissue (Fig. 3). The other implants continued to increase in size.

Because of the persistent frontal headaches and progressive left hemiparesis, the patient was readmitted to the Manhattan Veterans Hospital on April 8, 1958. Examination showed left hemiparesis, unsteadiness of gait, dysarthria, and poor memory. The tumor implants

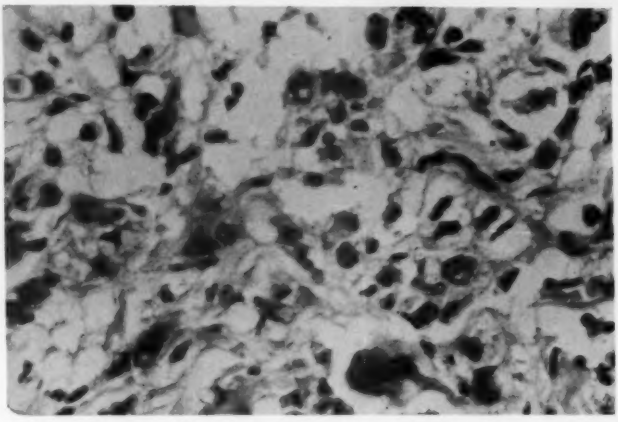

Fig. 3. Implant No. 3. Brain tumor tissue in subcutaneous tissue of anterior right thigh ninety-eight days after autotransplant. Shows typical anaplastic astrocytoma histology. × 750

in the thigh formed solid, circumscribed, subcutaneous masses which were movable (Fig. 4A).

The patient's symptoms and signs progressed, and left hemiplegia developed, with loss of pain, temperature, and position sense on the left. Hyperactive deep reflexes and a left Babinski sign were present, and the papilledema had increased. On April 18, 1958, the patient became disoriented and lethargic within twenty-four hours, and operation for decompression was performed. At operation, a large cystic cavity filled with fluid and tumor tissue was found in the right frontal parietal area and was partially resected. It was clear that the tumor had extended deep into the brain. Postoperatively, the patient was more responsive, but the left hemiplegia did not improve.

During the period of April 25 to July 22, 1958, the implants in the subcutaneous tissue of the thigh were biopsied several times. On May 8, two hundred sixty-six days after implantation, a small amount of tissue was taken from implant No. 5 for biopsy (Fig. 4A). This

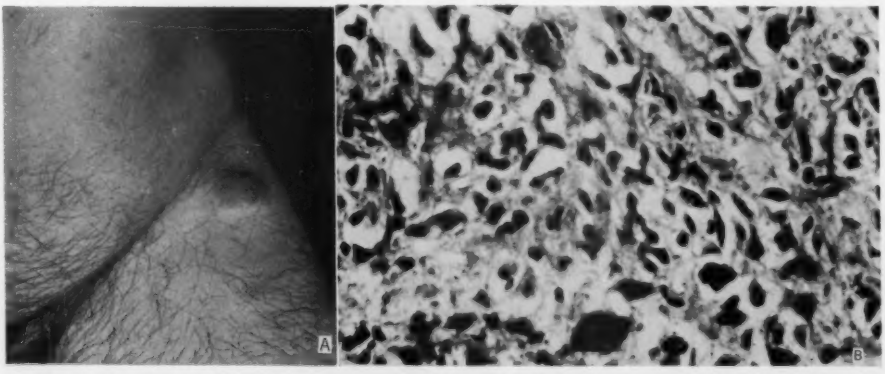

Fig. 4. [A] Implant No. 5. Brain tumor tissue implant in subcutaneous tissue of left thigh two hundred sixty-six days after implant. Before irradiation. [B] Implant No. 5. Brain tumor tissue in subcutaneous tissue of left thigh (see Fig. 4A) two hundred sixty-six days after implantation and before x-ray therapy. Typical anaplastic astrocytoma histology. × 750

revealed an actively growing anaplastic astrocytoma. Most of the cells were small, dark, and quite irregular, but a moderate number of large, bizarre, and almost giant forms was evident (Fig. 4B). The tumor cells were present among broad septa of dense connective tissue, and endothelial hyperplasia was minimal.

On May 15, x-ray therapy was given to implant No. 5; 120 kv, free aluminum filtration, and a portal of 5 cm. were used. The total dosage was 7,080 r (air-skin) and was given in 12 doses. This therapy was completed on June 2, and, on June 9, lesions No. 1 and 5 (Fig. 2) were excised, the latter after irradiation.

Histologic examination of lesion No. 1, that which had not received radiation, revealed large numbers of neoplastic cells highly variable in appearance; many were small and dark. Large, bizarre, nucleated cells were present as well. Many mitotic figures were noted. These cells were dispersed in a densely collagenous tissue. Large zones of necrosis were present as well as a slight inflammatory reaction. The endothelial cells of the blood vessels showed appreciable hyperplasia.

Histologic examination of implant No. 5 (Fig. 5B), after radiation, revealed an actively growing nodule of anaplastic tumor tissue in the corium of the skin. The tumor was very anaplastic and composed largely of rows of large tumor cells. Numerous mitotic figures were evident. The smaller dark cells, so common in the mass on the right side (nonradiated) and in the implant examined before radiation, were relatively absent from this mass, the cells being larger and more bizarre. The tumor nodule seemed to form a more homogeneous mass with a lesser tendency to infiltrate widely into adjacent structures, although such infiltration was unquestionably present. The degree of inflammatory reaction was also less here than in the nonradiated tissue on the right. Areas of necrosis were present, and some of these appeared to be undergoing organization. Adjacent portions of the corium were seen in which only a few scattered tumor cells were present. These areas showed an appreciable infiltration by inflammatory cells. In one area, the tumor cells appeared to have infiltrated into a small vein. Some of the cells in the deeper layers of the epidermis seemed quite irregular and bizarre, although they showed no tendency toward infiltration. Small subepidermal vesicles were present in some places, with a diffuse inflammatory infiltrate beneath these vesicles. The degree of vascular hyperplasia in this section was slightly less than that observed in nodule No. 1.

In mid-July, 1958, the patient became more lethargic, and his clinical condition was very poor. He died July 22. Necropsy showed a large cystic cavity in the right frontal area which measured 3 x 3 x 4 cm. in diameter.

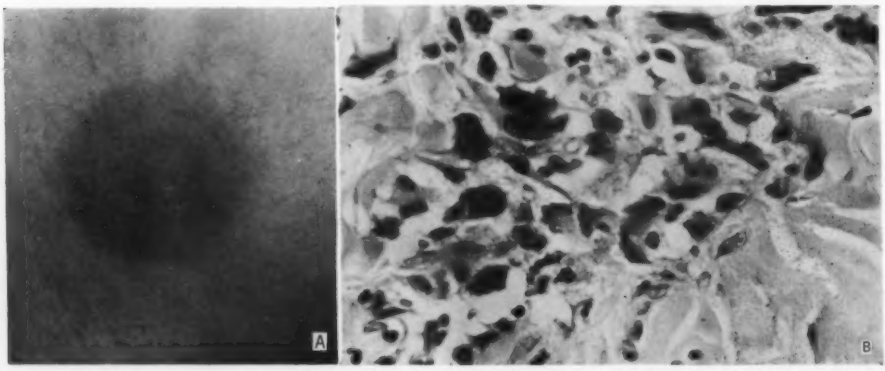

Fig. 5. [A] Implant No. 5. Brain tumor tissue in subcutaneous tissue of left thigh. After irradiation, 7,080 r (air-skin). [B] Implant No. 5. After irradiation, shows typical anaplastic astrocytoma histology. × 750

An irregularly outlined tumor, measuring about 5 cm. in diameter, involved the tissues immediately behind this cyst. The tumor extended from the wall of the cavity to the wall of the lateral ventricle. It was irregular and dirty gray in color and contained areas of small focal hemorrhage and areas of necrosis. Most of the tumor was located in the upper part of the right temporal lobe and extended from the lateral margins of this tissue to the corpus striatum and the thalamus on the right side. Multiple sections of this tissue demonstrated pleomorphic, bizarre, large cells arranged irregularly. The endothelial cells of the blood vessels were swollen and large. There were multiple areas of necrosis, cystic change, and hemorrhage.

DISCUSSION

In the case presented, the first evidence of implant growth was found thirty-four days after the autotransplant of the anaplastic astrocytoma in the subcutaneous tissue of the thighs. These implants continued to grow until the patient's death eleven and one-half months after transplantation. General autopsy was done, and no metastases were found.

At present, the efficacy of any treatment for brain tumors in man has to await clinical developments, which often require a long period of time for evaluation. This technic of tumor implant affords a rational approach to the study of various forms of anticancer therapy against brain neoplasms in man, and such studies have been initiated. The local or systemic injection of specific anticancer drugs could be done in vivo and the effects on the tumor implants studied by frequent and easy biopsy.

Various types of radiations, such as x-rays, proton emission, and radio frequency, could be tested by local exposure of the tumor tissue implants. Since many implants are available, it would be practical to evaluate, in a short period, several forms of therapy to determine the most effective treatment.

SUMMARY

1. Autotransplants of anaplastic astrocytoma into the subcutaneous tissues in man have been successfully grown; the histologic characteristics found in these implants are similar to the original brain tumor.

2. Specimens of such implants were easily biopsied, and the response to x-ray therapy has been studied.

3. Such subcutaneous implants of brain gliomas could be used to determine, in a short period of time, the most effective form of therapy against the specific cancer being studied. Therapy might include drugs and various forms of radiation.

The authors wish to thank Dr. Jerome M. Schacter for his help in several biopsies.

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Effect of trypan red on Columbia-SK virus pathogenicity

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THE EFFECTIVENESS OF TRYPAN RED in lowering the permeability* of the blood-brain and blood-cerebrospinal fluid barriers has been studied when the permeability of these barriers was abnormally increased under certain conditions of interest from a clinical standpoint.[1-6] Since trypan red and the closely related supravital dye, brilliant vital red, intensely stained the endothelial cells of the cerebrovascular system but did not permeate into the cerebrospinal fluid or the cellular masses of the brain, it was concluded that the endothelium constituted at least one important component of the blood-cerebrospinal fluid and blood-brain barriers.[1,3] In the clinically oriented studies mentioned, the importance of the blood-brain barrier as an underlying neurophysiologic mechanism, capable of conditioning the physiology and presumably the homeostasis of the central nervous system, was studied, and the role of endothelium in this was determined by studies in which trypan red was used, as compared with studies without trypan red.

In one study utilizing trypan red and other methods of altering the permeability of the blood-brain barrier, the possible relationship of the paretic involvement of anterior poliomyelitis to an increased permeability of the blood-brain barrier as a complication of this infectious process was examined.[7] Macaque monkeys were infected by the intranasal inoculation of the MV strain of poliomyelitis virus. The degree of paretic involvement was determined in control groups (inoculated and uninoculated with MV strain of poliomyelitis virus) and in corresponding groups of monkeys "protected" by trypan red alone and in combination with other methods. The clinical observations on the animals were amplified by pathologic studies on the cortex, diencephalon, and spinal cord, and determinations of blood-brain and blood-spinal cord permeability were made in which the distribution of cocaine hydrochloride, used as a tracer agent, was followed by spectrochemical methods, which permitted the identification and quantification of the tracer after its intravenous injection.

The theoretic basis for this initial study considered:

1] The likelihood of an increased permeability of the blood-brain barrier in the regions of the anterior horns of the spinal cord, in view of the known inflammatory reaction and swelling of the cord in poliomyelitis, the unique perivascular pathology associated with poliomyelitis, and the studies of MacCurdy and Evans,[8] which had suggested an altered permeability in poliomyelitis

2] The high vascularity of the anterior horns and physiologic evidence which indicates the high metabolic requirements and

*Permeability, as used in blood-brain barrier studies, refers to the permeation of tracer agents from the blood stream into the brain and not permeability in the classic physiologic sense. The uncertainty of the locus of the blood-brain barrier, the complexity of this barrier, and the likelihood of diffusion, as well as other mechanisms involved in addition to cell-membrane permeability, preclude a simple explanation in terms of any single structure or mechanism.

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Dr. Siegel's present address: Professor of Pathology, University of Oregon Medical School, Portland.

dependence of the anterior horn cells upon oxygen and other metabolites supplied by the vascular system of the spinal cord

3] The assumption that the difference between paretic and nonparetic involvement from individual to individual and at different levels and on different sides at the same level in the same individual might be explained in part or wholly by differences in permeability and the local alterations of pathophysiology that might be expected to accompany such differences of permeability. Local increases of permeability, associated with inflammatory reactions with their congestive infiltration of protein and other elements from the blood stream, might well result in both mechanical and physiochemical effects which could be deleterious to anterior horn cell function and survival.

Due to the considerable variations of permeability and paretic involvement encountered in these studies, it became obvious that the relationship between these two variables could be determined only by statistical methods, utilizing large control and test groups of animals. Because our facilities did not permit such large-scale studies on monkeys, this approach was abandoned. Nevertheless, the evidence adduced in this initial study tended to corroborate the thesis postulated. In the final report[7] to the grant-supporting agency, the National Foundation for Infantile Paralysis, the following conclusions were presented: "In summary, the spectrochemical data showed that, of the three tissues of the central nervous system studied, only the permeability of the blood-spinal cord barrier was lowered by treatment with trypan red in the poliomyelitis-infected group. The evidence . . . however, cannot be regarded as conclusive in that the difference between control (infected but not 'treated') and 'treated' (also infected) was not great. The clinical observations on the monkeys and neuropathological results may be regarded as suggestive of a 'protective' effect in the monkeys treated with trypan red. . . . Considering the evidence from these three lines of study, it would appear that the permeability of the cerebrospinal vascular tree may play a role of some importance in determining the perivascular pathology and paresis of poliomyelitis." ("Treated" refers to previous injections of trypan red.)

With the advent of technics which permitted a statistical evaluation of the results of virus infection on mice, the protective effect of trypan red against the neurotropic virus MM and Russian spring-summer[9] encephalitis was undertaken with negative results. However, in view of the suggestive results obtained in the preliminary poliomyelitis experiments on monkeys, a reexamination of this problem appeared warranted in experiments which utilized viruses of the mouse poliomyelitic strain.

Because mortality rate constitutes a more definite end point than paretic involvement in such studies on mice and because presumably there is a reasonably close relationship between paretic involvement and mortality, the latter was adopted in the present study.

METHODS AND RESULTS

Effect of trypan red. The virus used was the Columbia-SK strain of the encephalomyocarditis group.[10] The procedure consisted of inoculating a dose of 0.1 cc. of a sterile 1% solution of trypan red subcutaneously into 4- to 5-week-old Webster-Swiss strain mice on four consecutive days, after which no further trypan red was administered. On the fifth day, the mice received an intraperitoneal inoculation of 0.1 cc. of a 10^{-7} dilution of virus-infected mouse brain suspension. This dose was titrated to approximate the LD_{50} dose level, optimal for this type of study. As shown in the final tests (Table 1), an over-all mortality of 80 mice out of 165 tested was obtained in the control experiments by the dilution of the virus suspension used. A second group of mice, which had not been pretreated with trypan red,* was inoculated concurrently with a similar virus dose. All animals were then observed daily for fourteen days. A comparison of the results obtained in 6 separate experiments is shown in Table 1. As noted, the over-all percentage of animals receiving trypan red that were "protected" was approximately 50%. This evidence would appear to indicate that administration of trypan red conferred considerable protective effect against Columbia-SK virus.

*Subcutaneous injections of 0.1 cc. of normal saline were not done in this group to correspond with the subcutaneous injections of 0.1 cc. of a 1% solution of trypan red in the first group, in view of the well-known failure of such injections to produce physiologic effects. Also, see results of control injections of physiologic saline in Table 4.

TABLE 1

EFFECT OF TRYPAN RED IN MICE INFECTED BY COLUMBIA-SK VIRUS INTRAPERITONEALLY*

Experiment	*Virus only*	*% Death (a)*	*Virus plus trypan red*	*% Death (b)*	*% "Protected" ‡* $\left(\frac{a-b}{a}\right)$ 100
1	22/25†	88.0	13/25†	52.0	40.9
2	11/25	44.0	4/24	16.7	62.0
3	8/30	26.7	3/30	10.0	62.5
4	20/25	80.0	13/40	32.5	59.4
5	7/30	23.3	3/30	10.0	57.1
6	12/30	40.0	3/30	10.0	75.0
All experiments	80/165	48.5	39/179	21.8	55.1

*0.1 cc. of 1% trypan red subcutaneously for four days before intraperitoneal inoculation of 0.1 cc. of a 10^{-7} dilution of virus-infected mouse brain suspension on the fifth day

†Fatality rate = $\frac{\text{number of dead}}{\text{number of inoculated}}$

‡Protected = $\frac{\text{change in fatality rate}}{\text{control (that is, virus only) rate}}$

In another experiment, mice were inoculated by the intracerebral route with 0.03 cc. of a $10^{-7.5}$ dilution of virus suspension, after having received trypan red injections, as described in the previous experiment. The results are shown in Table 2. In neither instance was there a significant difference ($P > .50$ by χ^2 test) in the per cent mortality of animals treated with trypan red, as opposed to the controls. If experiments 1 and 2 are combined, it will be seen that increase of survival of only 1 mouse out of 60 was obtained in the groups of animals receiving trypan red, as compared to those that did not receive trypan red. This lack of a protective effect would be consistent with the intracerebral route of inoculation used, which bypasses the blood-brain barrier.

Effect of cortisone and trypan red. A total of 7 groups of 30 mice each was used in this study. The first group of mice was injected with 0.1 cc. of a 1% trypan red solution subcutaneously on four consecutive days, and, on the fifth day, 0.1 cc. of $10^{-6.5}$ suspension of the virus was inoculated intraperitoneally four hours after intraperitoneal administration of 0.04 cc. (1.25 mg.) of cortisone acetate. The other 6 groups of 30 mice each which were studied included the following: one group received the virus only; a second group received trypan red alone; the third group received cor-

TABLE 2

EFFECT OF TRYPAN RED IN MICE INFECTED BY COLUMBIA-SK VIRUS INTRACEREBRALLY*

Experiment	*Virus only*	*% Death (a)*	*Virus plus trypan red*	*% Death (b)*	*% "Protected" ‡* $\left(\frac{a-b}{a}\right)$ 100	*Significance test (x^2)*
1	18/30†	60.0	16/30†	53.3	11.2	0.27
2	23/30	76.7	24/30	80.0	–4.1	0.28

*0.1 cc. of 1% trypan red subcutaneously for four days before intracerebral inoculation of 0.03 cc. of a $10^{-7.5}$ dilution of virus-infected mouse brain suspension on the fifth day

†Fatality rate = $\frac{\text{number of dead}}{\text{number of inoculated}}$

‡Protected = $\frac{\text{change in fatality rate}}{\text{control fatality rate}}$

tisone alone; the fourth group received trypan red and the virus; the fifth group received trypan red and cortisone; and the sixth group received the virus and cortisone. The results of these studies are summarized in Table 3.

Trypan red afforded a "protected" percentage of 58.9 in the mice infected with virus in this experiment (Table 3, b), while cortisone increased the number of deaths (Table 3, c). Furthermore, cortisone administration resulted in obviating the protective effect of trypan red, so that the number of animals succumbing in this experimental group was similar to the number dying among animals receiving virus only (Table 3, d).

If the over-all mortality rate of animals inoculated with the virus alone in the initial experiment (80 of 165, or 48.5% from Table 1) is used instead of 56.7% (Table 3, a, based on 17 of 30), the percentage "protected" when trypan red was used (b) would be 52%, and the increased mortality with cortisone (c) would be −71.8%. When both trypan red and cortisone were used in conjunction with inoculations of the virus (d), the mortality, figured on this same basis, would be −16.9%, which suggests that the increased mortality resulting from the use of cortisone usually more than neutralizes the "protective" effect of trypan red.

Effect of dehydration and trypan red. In an experiment carried out to determine the influence of dehydration on the protective effect afforded by trypan red, infectivity of the virus in animals deprived of water was observed to be similar to that in the water-fed mice. Trypan red was administered to 50 mice that were subsequently inoculated intraperitoneally with Columbia-SK virus, as previously described. After virus inoculation, 25 mice were deprived of water completely for three consecutive days and then allowed to drink for one-half to one hour in the morning during the remainder of the experiment; 25 mice were allowed free access to water. The protective effect of trypan red was similar in both groups. In corollary controls of infected animals not given trypan red, dehydration was without any apparent influence on the course of infection. An additional control was employed in which 0.1 cc. of a sterile physiologic saline solution was injected subcutaneously on four consecutive days before intraperitoneal inoculation of virus on the fifth day. The difference in fatality rate from animals receiving no treatment (that is, no trypan red and virus only) before infection was negligible; in this instance, dehydration also was without any apparent influence on the course of infection (Table 4).

DISCUSSION

Various theories have been advanced with respect to the site and mode of action of the

TABLE 3

EFFECT OF CORTISONE ON PROTECTIVE EFFECT OF TRYPAN RED IN MICE INFECTED BY COLUMBIA-SK VIRUS INTRAPERITONEALLY*

Type of injected solution	*Fatality rate* $\left(\frac{\text{no. dead}}{\text{no. inoculated}}\right)$	*% Deaths*	*% "Protected"†* $\left(\frac{a-x}{a}\right)100$
Virus only	17/30	(a) 56.7	
Trypan red only	4/30	13.3	
Cortisone only	5/30	16.7	
Trypan red and virus	7/30	(b) 23.3‡	58.9
Trypan red and cortisone	5/30	16.7	
Virus and cortisone	25/30	(c) 83.3§	−46.9
Virus, cortisone, and trypan red	17/30	(d) 56.7	

*0.1 cc. of 1% solution of trypan red injected subcutaneously for four days before intraperitoneal inoculation of 0.1 cc. of a $10^{-6.5}$ dilution of virus-infected mouse brain suspension; 0.04 cc. (1.25 mg.) of cortisone acetate was injected intraperitoneally four hours before injection of virus suspension.

†Where x represents values shown under (b), (c), or (d)

‡Significantly different from 17/30 at 1% level

§Significantly different from 17/30 at 5% level

TABLE 4

EFFECT OF DEHYDRATION ON PROTECTIVE EFFECT OF TRYPAN RED IN MICE INFECTED BY COLUMBIA-SK VIRUS*

Treatment regimen	H_2O		Dehydration	
	Fatality rate†	% Deaths	Fatality rate†	% Deaths
Virus only	23/25	92%	24/25	96%
Physiologic saline‡ and virus	22/25	88	22/25	88
Trypan red	13/25	52	13/25	52

*0.1 cc. of 1% solution trypan red subcutaneously for four days before intraperitoneal inoculation of 0.1 cc. of 10^{-7} dilution of virus-infected mouse brain suspension on fifth day

†Fatality rate $= \frac{\text{number of dead}}{\text{number of inoculated}}$

‡0.1 cc. of sterile physiologic saline solution injected subcutaneously on four consecutive days before intraperitoneal inoculation of virus on fifth day

blood-brain barrier, and this subject has been extensively reviewed by several authors.[11–13] In order to explain the known differences between the blood-brain barrier and the blood-cerebrospinal fluid barrier, Aird and Strait[3] suggested that the cell membranes of the neuronal elements of the central nervous system were probably involved. Other theories have proposed that the barrier resides in the basement membrane of the capillaries[14] and in the glial membrane,[15] which in large part surrounds the capillaries of the central nervous system. The fact remains that present methods of study are not adequate to differentiate between the relative permeabilities of these more central structures. Because the endothelium constitutes the one component of the blood-brain barrier which can be identified and the permeability of which can be studied with relative precision by supravital dye and spectrophotometric technics, it was selected as a method of choice to be used.

In blood-brain barrier studies, it is essential that bonding effects between the tracer agent (in this case, the virus) and trypan red be ruled out. This was done in earlier studies when a closely related supravital dye, brilliant vital red, was shown not to be chemically bound by pharmacologic, dialytic, electrophoretic, and spectrographic methods after being mixed with such test agents as cocaine hydrochloride and strychnine.[1] However, because the supravital dyes of the diazo series may be bound in vivo by tissues and albumin, the possibility of bonding with viruses cannot be ruled out. Incubation of the virus in media containing dilute concentrations of the dye was not undertaken, owing to the well-known effect of incubation per se in altering the pathogenicity of viruses. Thus, although a bonding effect cannot be completely ruled out, the background of other studies referred to has made us favor the concept of a "blood-brain barrier" effect. The fact that similar virus studies[9] showed no "protective" effect when trypan red was used also contributed to our belief that a "blood-brain barrier" effect was involved rather than a bonding between the supravital dye and virus.

On the assumption that the effect noted is concerned with the "blood-brain barrier," the protective effect of trypan red against the intraperitoneal inoculation of Columbia-SK virus in mice, as demonstrated in this study, might be explained in one of two ways or possibly by a combination of both. Since trypan red did not materially protect against the virus when inoculated intracerebrally, it might be concluded that the effect of the trypan red was to lower the permeability of the blood-brain barrier to the virus. The demonstrated effect of trypan red on the permeability of the cerebrovascular system in a series of previously reported studies[3,4,6] would be in keeping with this concept.

However, in keeping with the thesis postulated in the introduction of this report, a second explanation might also be considered. This was suggested in the case of anterior poliomyelitis by the known ubiquity of the virus-

es causing this disease during epidemics and their relative inocuousness in the great majority of patients. The neuropathology of this condition and other factors discussed suggested the possibility that pathophysiologic mechanisms associated with an increased permeability of the blood-brain barrier might be responsible for the more serious complications of the disease.

Since such secondary mechanisms might be expected to bear some relationship to the concentration of the virus gaining entrance to the parenchyma of the central nervous system, as well as to the virulence of the virus, it would seem likely that both explanations might be involved.

The failure of trypan red to afford statistically significant protection in the earlier experiments with the neurotropic viruses MM and Russian spring-summer encephalitis would appear to fit in better with the second explanation presented and also suggests that differences in the results obtained with various viruses might be expected, depending upon such factors as the acid-base dissociation characteristics of the virus. As suggested by Friedemann,[16] the blood-brain barrier is relatively impermeable to electronegative substances, including several viruses, while electropositive substances permeate the barrier with ease. This electrochemical concept has been substantiated in studies by Fisher and associates[17] and our own group,[18] who have shown that the acidic dissociation (pKa) of the sulfonamides bore an important relationship to the permeation of these compounds through the blood-brain barrier.

On the basis indicated, it might be postulated that, when the infectious process attains major proportions and produces serious secondary effects in the central nervous system, permeability factors might assume great importance and at least in part explain some of the more serious complications of virus diseases. Because the paretic effects of poliomyelitis constitute the most important and dreaded complication of this disease, it would be of some little theoretic, if not practical, importance to extend these studies more specifically to the protection that might be afforded by trypan red against the paralytic complications of anterior poliomyelitis. Correspondingly, the possible protective effect of trypan red for other viruses might likewise be investigated.

The increased mortality of the mice to Columbia-SK virus when treated with cortisone would be in keeping with the deleterious effect of this hormone in such infections as tuberculosis[19,20] and in a number of virus infections.[21,22] The apparent relationship between cerebrovascular permeability and virus infectiousness might be explored by utilizing other mechanisms which increase or lower the permeability of the blood-brain barrier. The primary or secondary importance of such mechanisms in infectious processes of the central nervous system may be considerable, judging by the evidence of this study and certain other studies which might be interpreted as involving permeability changes, such as trauma[23,24] and dental extraction,[25,26] which have been advocated as precipitating mechanisms for infection.

SUMMARY AND CONCLUSIONS

Preliminary trypan red injections reduced the fatality rate in mice more than 50% on the average when Columbia-SK virus was subsequently inoculated intraperitoneally but afforded no protection when the inoculations were given intracerebrally. In view of other studies on the effect of trypan red on the permeability of the endothelial component of the blood-brain barrier, this result was interpreted as a protective effect in terms of diminished blood-brain barrier permeability to the virus and/or the central effects of such diminished permeability on pathophysiologic factors affecting survival.

Cortisone increased the mortality and nullified the protection afforded by trypan red in mice inoculated intraperitoneally with Columbia-SK virus. Dehydration failed to increase or diminish significantly the protective effect of trypan red in similar experiments.

Further studies to determine the possible protection of trypan red against the paretic effects of poliomyelitis and the possible beneficial effects of trypan red in other virus diseases appear indicated.

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Immunoelectrophoretic studies of cerebrospinal fluid

Investigations in patients with tumor in the central nervous system

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DURING THE LAST DECADE, a number of new methods have been elaborated for separating proteins in biologic fluids. Most of these methods utilize the principles of electrophoresis and, during the last years, particularly immunoelectrophoresis. Investigations using these new technics have widened our knowledge of the plasma proteins,[1–3] while corresponding analysis of cerebrospinal fluid has been difficult due to its relatively small protein content.

The immunoelectrophoretic method described by Grabar and Williams,[4] and by Scheidegger[5] as a micromethod, provides new possibilities of studying the composition of the cerebrospinal fluid proteins and therefore offers new diagnostic possibilities.[6,7] Until a few years ago, the only routine clinical methods available for examination of the cerebrospinal fluid were those for determining the total protein, colloid reactions, and paper electrophoresis. The information obtainable by such methods has proved of little value in the diagnosis of tumors of the central nervous system. With paper electrophoresis, it is possible to determine 7 groups of proteins in the cerebrospinal fluid. Immunoelectrophoresis, however, implies a further advance because, unlike paper electrophoresis, it separates the individual proteins, and not simply groups of proteins, from one another. As yet, more than 30 different protein fractions have been separated in the spinal fluid by immunoelectrophoresis.[8]

METHOD

Immunoelectrophoresis is a two-stage method in which a physicochemical separation of the proteins is combined with an immunologic precipitation. The first stage consists of an electrophoretic separation of the various protein fractions in the solution studied and the second, of a diffusion of the electrophoretically separated fractions against antibodies from an antihuman serum according to the principles for Ouchterlony's[9] precipitation technic. Where antigen and specific antibody meet, there occurs a precipitation recorded by a more or less elongated arc, each corresponding to a separate protein.

The micromethod used consists of a modification[2] of that described by Scheidegger.[5] Some microliters of concentrated cerebrospinal fluid containing a standardized amount of protein are placed in a hole, 1 mm. in diameter, in an agar gel layer on an ordinary glass slide. After electrophoresis (three hours), the 7 main protein fractions are separated in the longitudinal direction of the glass in the usual way. Antihuman serum or specific antiserum (for example, antifibrinogen serum) is then placed in a groove cut in the agar gel in a direction parallel to the electric field. By diffusion for two days, antigen-antibody precipitates are formed, which appear as arcuate lines in the transparent agar film on the glass slide. These lines can be brought out more clearly by special staining methods (Fig. 1). The cerebro-

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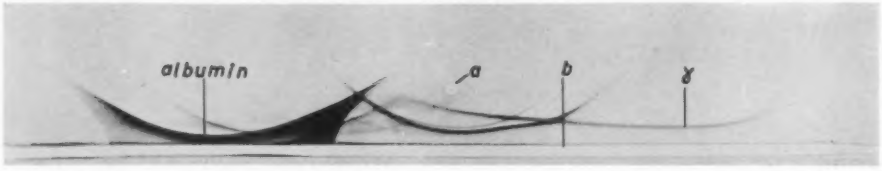

Fig. 1. Normal microimmunoelectrophoretic pattern of cerebrospinal fluid. To left, albumin fraction; to right, γ-globulin. [a] Application spot for protein sample. [b] Groove for antiserum

spinal fluid proteins hitherto separated[8] by immunoelectrophoresis are given in Figure 2.

In the investigation of patients with tumors, we paid special attention to some of the protein fractions common in such diseases but practically absent in spinal fluid from normal persons. These pathologic fractions are given schematically together with some normally occurring fractions for reference in Figure 3. The fractions to which special attention was given in the present investigation were:

α_2-macroglobulin, a spherical glycoprotein with a molecular weight of 846,000. As mentioned previously,[10] it has been possible to demonstrate α_2-macroglobulin with the aid of anti-α_2-macroglobulin serum in spinal fluid samples from most patients without neurologic disorders. If antihuman serum is used instead of anti-α_2-macroglobulin serum, the fraction will not be demonstrable so often, suggesting that precipitation requires a larger amount of the α_2-macroglobulin. In the present investigation, only antihuman serum was used, so that the α_2-macroglobulin arcs seen mean a relatively high concentration of this protein. A very high concentration of the α_2-macroglobulin, resulting in an arc of serum type, is never obtained in spinal fluid samples from those without neurologic signs, however.

β_1-lipoprotein, a spherical molecule with a molecular weight of 1,300,000. This protein was never demonstrable in cerebrospinal fluid samples from normal persons.[11] It is best demonstrated with lipid stains (Sudan black).

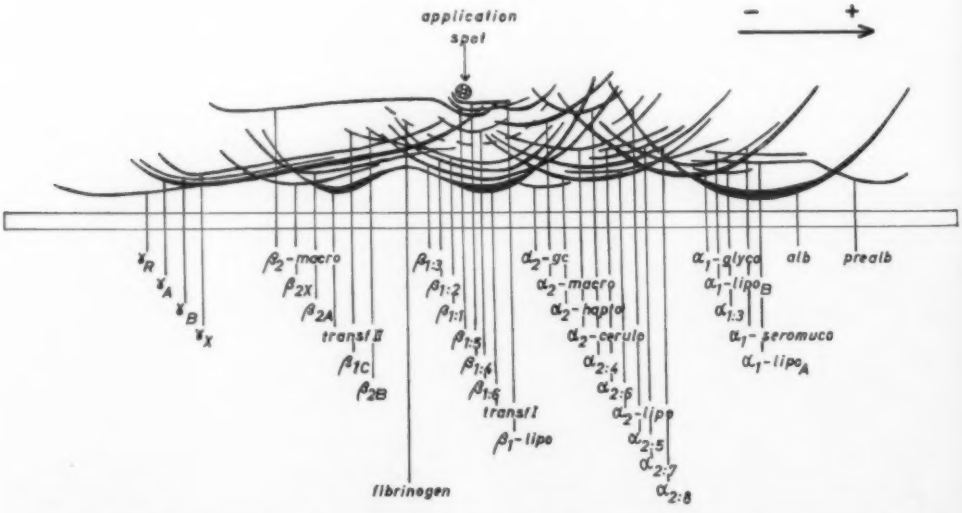

Fig. 2. Model showing all cerebrospinal fluid proteins demonstrated with antihuman serum and antifibrinogen serum

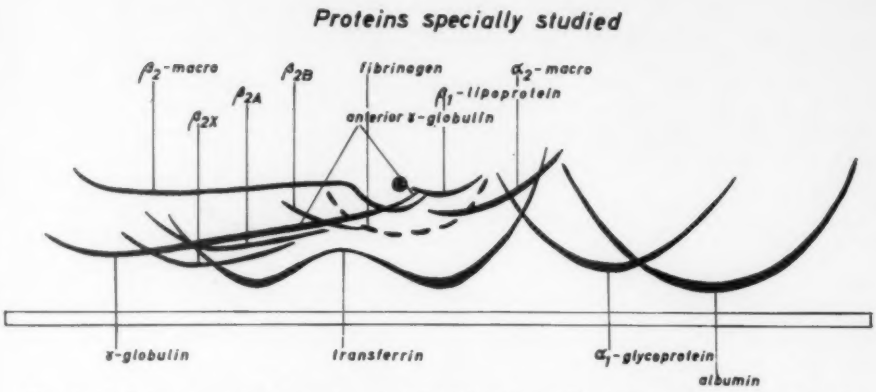

Fig. 3. Pathologic proteins in cerebrospinal fluid (top) specially studied in this paper. For reference: 4 normal main fractions (bottom)

Fibrinogen, a long ellipsoid molecule with a molecular weight of 330,000. It has not been demonstrable in normal spinal fluid[12] and is precipitated with a special antifibrinogen serum.

β_2-globulins. Of these, β_{2A}-, β_{2B}-, β_{2M}-, and β_{2X}-globulin could not be demonstrated in normal spinal fluid by the standardized immunoelectrophoretic method described above. Only little is known of their properties. According to Heremans,[2] they may be regarded as immunoglobulins.

γ-globulin. In normal cerebrospinal fluid, the anterior, large molecular part of the precipitation line of this protein is missing or occurs only in the form of a weak precipitate. But this part of the line was found to be marked in many patients with neurologic diseases and is therefore recorded as a special finding.

MATERIAL

The material consisted of cases of brain tumor and tumor of the spinal cord and meninges seen at the University Hospital, Lund, in 1960. With few exceptions, all the patients had been operated upon and were thus definitely examined cases. A firm pathologic diagnosis had been made in 40 of the 46 cases. Cases in which the diagnosis of tumor was uncertain were not included. The material is summarized in Table 1.

The control group was 30 patients who had sought advice for headache, dizziness, and mild mental symptoms. None of them had symptoms or signs of a neurologic disorder. This control series cannot, of course, be regarded as real normal material. The use of such a control series does not, however, limit the value of the differences found between the patients with tumor and the controls because the difference found would probably have been even more striking if the controls had consisted of perfectly normal persons.

TABLE 1
TYPES OF TUMORS IN PATIENTS STUDIED

		Number of Cases
Cerebral tumor		
Malignant	19	
Glioma		11
Metastases		5
Other tumors		3
Benign	11	
Meningioma		2
Neurinoma		4
Hypophyseal adenomas		4
Other tumors		1
Spinal tumor	14	
Subdural with occlusion		4
Subdural without occlusion		6
Extradural with occlusion		3
Extradural without occlusion		1
Arteriovenous aneurysm	2	
Cerebral		1
Spinal		1
Total		46

TABLE 2

SUMMARY OF CEREBROSPINAL FLUID FINDINGS

Clinical group	Number of cases	Total protein*	Paper electrophoresis (%)		Immunoelectrophoresis							
			$\beta \geq 20$	$\gamma \geq 13$	Pathologic fractions (mean)	Alpha$_2$-macroglobulin	Beta$_2$-lipoprotein	Fibrinogen	β_{2M}	β_{2A}	β_{2X}	β_{2B}	Marked anterior γ
Control	30		6	7	0	8							
Cerebral tumor													
Malignant	19	8	1	8	3.6	10	11	10	6	6	3	2	7
Benign	7	5	1	2	5.0	5	5	4	3	3			2
Spinal tumor													
Without occlusion	7	1		1	5.0	7	5	5	2	3		2	4
Froin's syndrome													
Cisternal puncture	6	1	2	1	4.5	5	5	4	2	3		2	2
Lumbar puncture	7	7			5.8	6	7	7	1	5		2	4

* $\geq$ 60 mg. per 100 cc.

RESULTS

The total protein and the results of paper electrophoresis and immunoelectrophoresis are summarized in Table 2. The table includes the 30 controls, 26 patients with cerebral tumor (19 malignant, 7 benign), and 14 with spinal cord tumor (7 with and 7 without Froin's syndrome, cisternal and lumbar spinal fluids accounted for separately). The table does not include the 4 cases of pituitary adenoma, which showed a relatively normal picture, or the 2 cases of arteriovenous aneurysm, which showed a clearly pathologic picture. The values given in the table are not strictly comparable because the values are absolute and the groups are not of uniform size.

The third column of the table gives the patients in each group with increased total protein, that is, more than 60 mg. per 100 cc. About half of the tumor series (21 of 40) showed such an increase, while only a few of those with a nonoccluding spinal tumor showed high values.

The following columns give the number of cases in which paper electrophoresis showed increased β- and γ-fractions. The upper limits of the normal ranges of variation in this material were said to be 20 and 13%, respectively. In only a few of the tumor cases did the values exceed these limits. There was no true difference between the controls and the tumor cases.

The rest of the table shows the results of the immunoelectrophoretic studies. The first column gives the mean number of pathologic protein fractions found in the individual cases in the different groups. On the average, about 14 cerebrospinal fluid protein fractions were found in each of the "control" cases. In the tumor cases, we also found pathologic fractions in addition to these 14 normal fractions, on the average 3 to 6 in each case. This implies, for example, that the cerebrospinal fluid from patients with benign tumor of the brain or spinal cord have, on the average, 5 protein fractions more than can be demonstrated in subjects without tumor in the central nervous system. It might seem perhaps that the number of pathologic fractions was somewhat lower in the cases of malignant brain tumor than in those of benign brain tumor and tumor of the spinal cord. This might be explained by

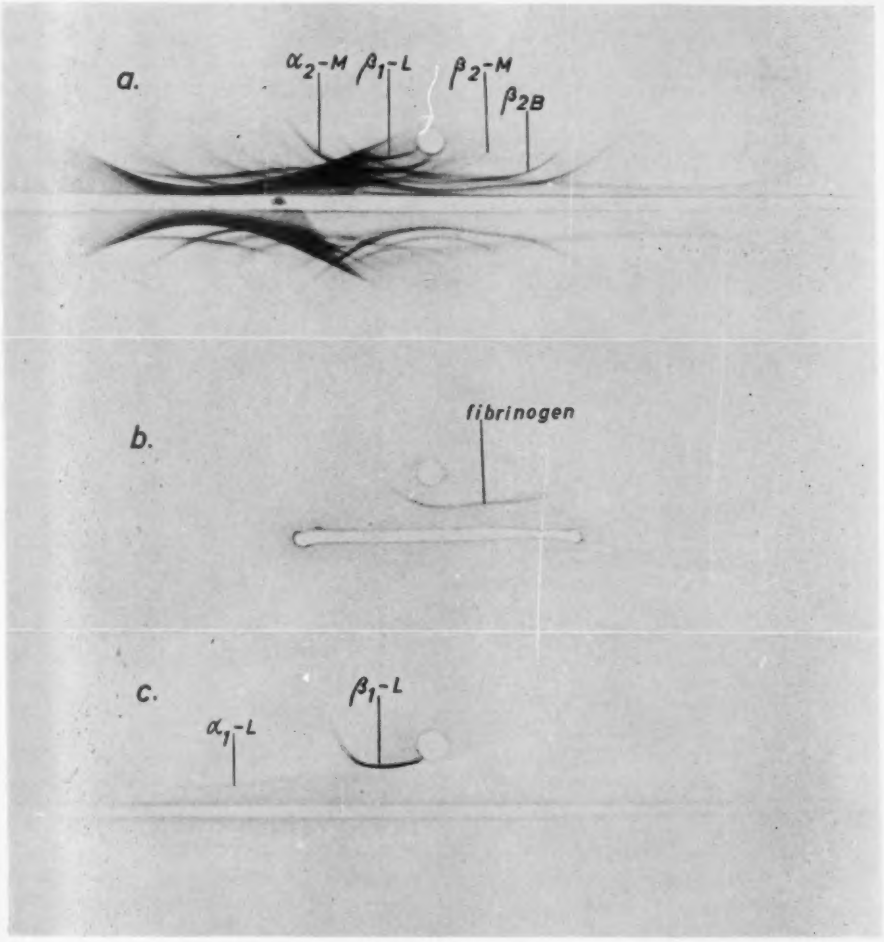

Fig. 4. Immunoelectrophoretic pattern (above groove) of cerebrospinal fluid from patient with malignant cerebral tumor (glioma) and from another patient (below) with benign tumor (pituitary adenoma). [a] After protein staining with amido black, marked β_1-lipoprotein (β_1-L) and α_2-macroglobulin (α_2-M), lines appear in malignant (upper) case. β_{2B}- and β_{2M}-globulin fractions are also seen. No such lines are seen in lower case in which protein pattern is almost normal. [b] Same cases after precipitation with antifibrinogen serum. Distinct precipitation line is seen in malignant (upper) case but not in lower. No other proteins were precipitated. [c] Same cases stained for lipids (Sudan black). Marked β_1-lipoprotein line is seen in malignant (upper) case and hardly discernible line in lower. α_1-lipoprotein (α_1-L)—a normal finding—is also seen. Other proteins were also precipitated but not stained.

the fact that the benign tumors are usually meningiomas or neurinomas and, owing to their intimate relation to the cerebrospinal system, might be able to affect the composition of the spinal fluid.

Some of the pathologic fractions in the

tumor cases have been noted in the following columns:

α_2-macroglobulin sometimes occurs in lower concentration in normal persons. It was thus found in only one-fourth of the controls (in 8 of the present 30 controls) but in three-fourths of the patients with tumors (28 of 40), and in these cases the arc was usually more marked, which it never was in the controls.

β_1-lipoprotein is a pathologic finding in the cerebrospinal fluid. It was never found in any of the controls but was found in three-fourths of the patients with tumor (28 cases).

Fibrinogen does not normally occur in the cerebrospinal fluid. On the other hand, two-thirds of the patients with tumor (26 cases) had fibrinogen in the cerebrospinal fluid, and in most of these cases the precipitation arc was marked.

In addition, **4β_2-fractions** were found. None of these fractions were demonstrable in any of the controls, but all 4 were found in anything up to one-third or more of the patients with tumor.

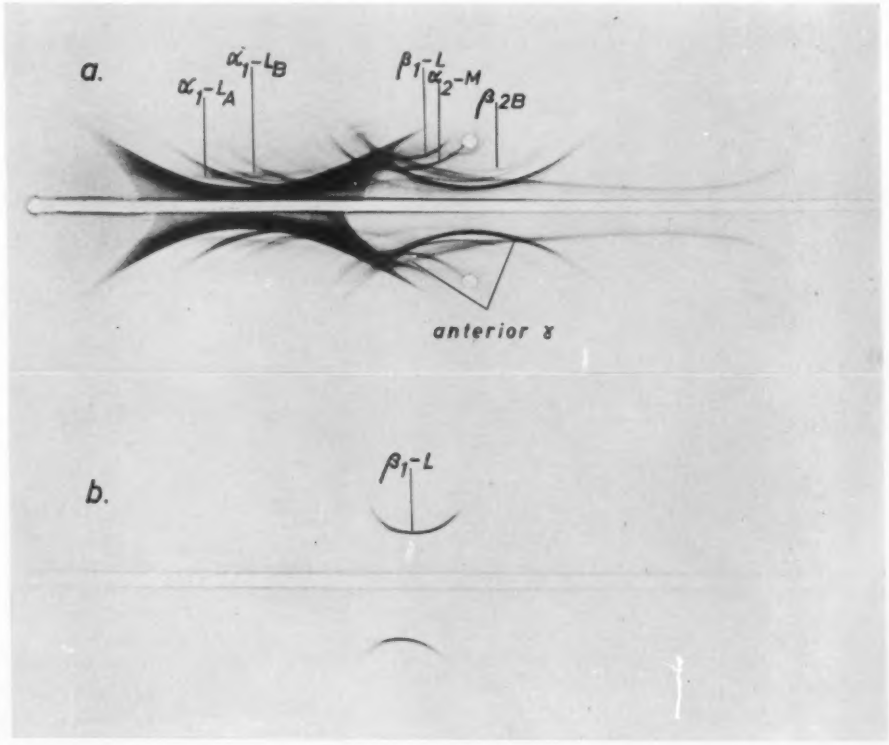

Fig. 5. Immunoelectrophoretic pattern of cerebrospinal fluid from patient with spinal cord tumor (meningioma) with Froin's syndrome: cisternal fluid above, lumbar fluid below. Concentration of proteins in lumbar fluid was 10 times higher than in cisternal fluid; both specimens were concentrated so that samples studied had same concentration of proteins. Though samples were obtained from 2 nonintercommunicating spaces and had quite different total protein content, immunoelectrophoretic pattern was almost same in both. [a] Protein staining with amido black. Observe double α_1-lipoprotein lines (α_1-L_A and α_1-L_B), marked β_1-lipoprotein (β_1-L) and α_2-macroglobulin (α_2-M) lines, marked anterior parts of γ-globulin, β_{2B}-globulin, and transferrin arc of serum type. [b] Lipid staining (Sudan black). Marked β_1-lipoprotein lines in both samples.

Finally, no patient in the control group was found to have a **marked anterior γ-globulin line,** that is, an increased concentration of the large molecular part of the γ-globulin. We found this marked fraction in almost half of the patients with tumor (17 cases).

The tumor series is exemplified by 2 illustrations of the results of immunoelectrophoresis in some typical cases. The first (Fig. 4) refers to a case of malignant cerebral tumor (glioma) compared with a benign tumor (pituitary adenoma); the other (Fig. 5) compares cisternal and lumbar fluid in a case of spinal cord tumor with total occlusion (Froin's syndrome).

SUMMARY AND CONCLUSIONS

Immunoelectrophoresis of the cerebrospinal fluid offers better possibilities of a more refined examination of the fluid for proteins. In the present investigation, spinal fluid proteins were studied by microimmunoelectrophoresis in 46 cases of cerebral and spinal cord tumors.

Examination by conventional methods (determination of total protein, colloid reactions, and paper electrophoresis) has, as a rule, proved to be of limited value in the diagnosis of such cases. The total protein was raised in half of the patients with tumor in this series, and paper electrophoresis showed no certain difference from the control series. Immunoelectrophoresis, on the other hand, showed not only the 14 proteins occurring in normal persons but also nearly always the presence of pathologic fractions. Thus, in the patients with tumor, the cerebrospinal fluid showed on the average 5 abnormal fractions; that from the 19 patients with malignant cerebral tumor, 3 to 4 pathologic proteins; and the 7 with benign cerebral tumor and the 14 with spinal cord tumor, 5 to 6 pathologic proteins. Fluid from the 4 patients with pituitary adenoma, however, showed essentially a normal picture.

Increased amounts of α_2-macroglobulin and β_1-lipoprotein were seen in three-fourths of the cases and fibrinogen in two-thirds; 4 different pathologic β_2-fractions were all found in anything up to one-third or more and an increased large molecular portion of the γ-globulin in almost half of the patients with tumor. These findings contrast sharply with what was seen in the controls. Many of the patients with tumor and pathologic protein fractions had shown a normal paper electrophoretic pattern.

No true difference was found between the findings in the patients with malignant and benign tumors, but the results tended to differ with the type of tumor, so that immunoelectrophoresis may be able to contribute to a more refined differential diagnosis of cerebral and spinal cord tumors. However, further investigation on a larger series is needed before any valid conclusion can be made in this respect.

The cerebrospinal fluid from patients with cerebral or spinal cord tumor was thus found to contain more protein fractions and to a large extent also pathologic fractions *(inter alia* β_1-lipoprotein, fibrinogen, and certain β_2-fractions), which never occur in normal persons. Among the 46 cases of tumor, the immunoelectrophoretic pattern of the cerebrospinal fluid proteins was normal in only 4 but pathologic in the remaining 42 (91%).

The authors thank Dr. Nils Lundberg and Dr. Stig Jeppsson of the Department of Neurosurgery at the University of Lund for kind cooperation and Miss M. Linell and Mrs. S. Ohlsson for technical assistance.

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Chronic cerebrospinal fluid otorrhea

Report of a case associated with chronic infection of the ear

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THE DISCHARGE of cerebrospinal fluid from the external auditory canal has seldom been reported except as a sequel of cranial trauma or surgery. In 1951, Nenzelius[1] described a case of spontaneous cerebrospinal fluid otorrhea associated with a congenital fistula. He could find only 2 similar accounts in the literature since 1879. Subsequently, in 1958, Filonenko[2] encountered a meningocele which lay in the tympanic antrum of an infant who presented with acute otitis, meningitis, and cerebrospinal fluid otorrhea.

In addition to these congenital lesions, spontaneous or nontraumatic cerebrospinal fluid otorrhea has been reported on 2 occasions as a complication of chronic aural infection. Canfield,[3] in 1913, described a 23-year-old man with a six-week history of pain in the ear and a one-week history of cerebrospinal fluid otorrhea who died shortly of purulent meningitis. Autopsy disclosed a large petrosal air cell containing pus and cerebrospinal fluid, the site of the fistula. Mosberg,[4] in 1959, described spontaneous cerebrospinal fluid otorrhea of twenty-four days' duration in a 63-year-old man with chronic otitis media. Endaural mastoidotympanectomy was carried out, and the fistula was repaired with a split-thickness skin graft to the dural defect.

The following case is presented as another uncommon example of spontaneous or nontraumatic cerebrospinal fluid otorrhea associated with chronic infection of the ear. It is unusual also in that the cerebrospinal fluid otorrhea was present intermittently for eight years before surgical repair.

CASE REPORT

The patient, a 69-year-old retired barber, was admitted to the Massachusetts General Hospital on February 15, 1960, acutely ill with purulent meningitis. Cerebrospinal fluid flowed profusely from his right external auditory canal.

Documented history went back to March 1952, when he was first seen at the Massachusetts Eye and Ear Infirmary with a complaint of painless, clear, watery discharge for ten days from the right external auditory canal, not associated with trauma, tinnitus, vertigo, or headaches. He vaguely recalled that some thirty to thirty-five years earlier he had received treatment for chronic ear disease at the Boston City Hospital, including surgery (myringotomy?). No record of any such treatment has been found, however, in a search of the files at that hospital. He apparently had no subsequent otic symptoms for which he sought medical treatment until 1952.

Otologic examination in March 1952 revealed on the right a thickened tympanic membrane with a posterosuperior perforation through which clear, watery, odorless fluid dripped into the external auditory canal. There was no evidence of previous retroaural or endaural mastoid or middle ear surgery. The left ear was dry with a scarred tympanic membrane. Roentgenograms disclosed bilateral, symmetrically underdeveloped mastoids without evidence of destruction. An audiogram revealed a slight conductive loss (0 to 15 db.) in the lower frequencies and a perceptive deafness in the higher frequencies, essentially the same in either ear and consistent with chronic middle ear disease.

The right ear was treated topically intermittently throughout 1952. On one occasion, it was noted that the Valsalva maneuver caused the clear fluid to squirt profusely out

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of the external auditory canal into the examiner's speculum. On another occasion, when the perforation of the right tympanic membrane had temporarily closed, it was reopened by carrying out the Valsalva maneuver; the examiner thereupon noted that a profuse jet of clear watery fluid squirted from behind the drum into the external auditory canal. A review of the hospital record, however, failed to show that a diagnosis of cerebrospinal fluid otorrhea was suspected.

The patient was not seen from November 1952 until October 1958, when he presented with the same complaints and findings. He stated that the watery drainage from his right ear had persisted intermittently during the preceding six-year interval. The perforation in his right drum closed again with cessation of otorrhea, but the discharge had recurred when the patient was seen in January, February, and September 1959. Cultures of the watery discharge taken in September grew out Staphylococcus albus on one occasion and Escherichia coli on another.

The patient was next observed when he was admitted in February 1960. His wife related that he had complained intermittently of headaches, chills, and fever during the previous week. Examination revealed the patient to be deeply stuporous, obeying no commands, with marked nuchal rigidity. The temperature was 101° F. There was a profuse discharge of clear, watery fluid from his right external auditory canal. This fluid escaped from behind an inflamed right tympanic membrane via a small perforation. Lumbar puncture disclosed an opening pressure of 250 mm. Examination of the cerebrospinal fluid revealed 3,000 white blood cells per cubic millimeter, 90% polymorphonuclears, and a sugar of 15 mg. per cent. No organisms were seen in a gram-stained smear of the sediment. The offending organism was never isolated from blood cultures or from cultures of the cerebrospinal fluid. However, Escherichia coli was cultured from the discharge from the right ear.

The patient was treated vigorously with meningitic doses of penicillin, Chloromycetin, and sulfadiazine, with complete clearing of the cerebrospinal fluid in his second hospital week. The watery discharge from his right external auditory canal persisted unabated and essentially continuously. Rough quantitative estimates of 100 to 300 cc. per day were recorded. Tests for sugar in this fluid were repeatedly positive. At the end of his second hospital week, the patient remained febrile, lethargic, and confused. Except for a persistent nystagmus with right lateral gaze, focal neurologic signs were absent.

Repeated radiologic examinations of the skull were performed in an attempt to demonstrate erosion or fracture of the petrous bone. All of these studies were interpreted as normal. On February 27, 1960, a Pantopaque lumbar encephalogram (1 cc.) was carried out in an attempt to localize the spinal fluid leak. The Pantopaque, however, remained in the basilar cisterns. These findings hinted that the craniootic fistula might be from the middle fossa.

Accordingly, on March 1, 1960, a small right temporal craniectomy was performed. The right temporal lobe was elevated extradurally. As the midportion of the petrosal ridge was exposed, multiple nubbins of extradural granulation tissue were encountered which were adherent to and extended into perforations of paper-thin areas of bone. These granulations were bluntly dissected free, and, as the temporal lobe was elevated more medially, a total of 6 perforations in the area of the tegmen tympani were noted. These bony erosions varied from 2 to 5 mm. in diameter and gave a "Swiss cheese" appearance to the tegmental portion of the right petrous bone. No gross defect in the dura was noted; however, droplets of cerebrospinal fluid seeped from the nubbins of granulation tissue on the dura. The perforated area of bone was covered with a layer of Gelfoam; 2 strips of fascia lata were sutured to the abnormal area of dura. The wound was then closed in anatomic layers without drainage.

Immediately postoperatively, the cerebrospinal fluid otorrhea ceased. Within forty-eight hours, the perforation in the right tympanic membrane was closed. The patient's postoperative recovery was otherwise prolonged by persistent lethargy and confusion. He also had intermittent, low-grade fever, gastrointestinal hemorrhage, and unexplained intractable diarrhea. He was discharged from the hospital on May 15, 1960.

By July 1960, the patient's neurologic and general physical recovery was complete. His hearing had improved by audiogram test over that recorded in 1952, with the right ear being the ear more nearly normal. When last examined at the Massachusetts Eye and Ear Infirmary in December 1960, the right tympanic membrane was intact. Correspondence in April 1961, some thirteen months after operation, disclosed that he has been getting along quite well and that there has been no further drainage from his right ear.

COMMENT

It seems improbable that the etiology of the cerebrospinal fluid otorrhea in this patient was traumatic, that is, from either a long-delayed complication of possible aural surgery performed thirty to thirty-five years earlier or a skull fracture. Otorrhea or otorhinorrhea seen after operation occurs promptly in the postoperative period.[3–7] Also, the fistulas in Dandy's[5] 4 cases of otorrhea after mastoid surgery were from single discrete defects in the subtemporal dura and petrous bone, 0.5 to 1 cm. in diameter. The descriptions of these defects do not resemble the operative findings in the patient reported here. Lastly, no history of head injury was ever obtained, and no fractures were seen roentgenologically or at operation.

That this patient had chronic aural infection is abundantly clear from the history and numerous examinations. This infection presumably originated as a childhood otitis media; it became quiescent on the left. As Cawthorne[8] has recently reiterated, bony erosion of the thin tegmen tympani is the route of spread of chronic infection from the middle ear to the middle cranial fossa; he further commented that this bony erosion may be a lengthy process, requiring years, before the infection affects the central nervous system. The operative findings in this case suggest such an inflammatory pathogenesis and exclude a congenital lesion, fistulous stalk, or meningocele. The multiple "Swiss cheese" perforations of the tegmen tympani with adherent nubbins of extradural granulations are consistent with chronic osteomyelitis of this portion of the petrous bone.

It seems eminently reasonable to attribute the sequence of events in this patient to chronic aural infection. This inflammatory process ultimately produced a craniootic fistula which discharged cerebrospinal fluid intermittently and profusely over a period of eight years before purulent meningitis occurred after an acute flare-up of the otitis. There may well be unreported cases of spontaneous cerebrospinal fluid otorrhea associated with chronic middle ear disease. However, despite numerous extensive surveys of the intracranial complications of chronic suppurative otitis media, the evidently rare association of cerebrospinal fluid otorrhea is not described.[9–12]

SUMMARY

An account is given of a patient with chronic ear infection who presented with cerebrospinal fluid otorrhea. After the otorrhea had persisted for some eight years in this individual, purulent meningitis developed. The causal craniootic fistula was subsequently identified during an exploration of his middle cranial fossa and was repaired (thirteen-month follow-up) with a fascia lata graft. After a study of the literature, it is suggested that this case is a singular example of prolonged cerebrospinal fluid otorrhea resulting from chronic infection of the ear.

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Hereditary interstitial nephritis associated with polyneuropathy

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THE PURPOSE OF THIS PAPER is to briefly describe the peripheral neurologic symptomatology encountered in two unrelated cases of hereditary interstitial nephritis recently studied in the Peter Bent Brigham Hospital. The neuropathologic changes observed in the peripheral nerves of one patient will be described, and an attempt will be made to assess the significance of both cases.

This rare condition, known as hereditary interstitial nephritis,[1] hereditary interstitial pyelonephritis,[2] hereditary familial hemorrhagic nephritis,[3] and congenital hereditary hematuria,[4] is characterized by the occurrence in members of a family of chronic progressive renal disease associated with bilateral nerve deafness. The hearing loss or the kidney lesion may appear as an isolated abnormality in some members of the family. These conditions tend to be progressive, and the majority of affected individuals whose cases have been reported died of renal insufficiency at a relatively early age. An extreme tendency to chronic renal infection and development of interstitial pyelonephritis constitutes a remarkable feature of the renal lesion. From the pathologic standpoint, atrophic, granular, retracted kidneys with chronic interstitial pyelonephritis, tubular atrophy, and a variable degree of glomerular involvement are usually found.

The pathologic changes in the ears have not as yet been fully studied.

Males are affected more seriously and females more frequently. The type of inheritance is that of a partially sex-linked dominant trait.[5] The syndrome has been found in various geographic areas and in patients of different racial extractions. In addition to the renal and hearing abnormalities, ocular defects have also been described. Sohar[6] refers to 2 cases with spherophakia (spherical lenses) and 2 cases with congenital posterior cataracts. One of our patients had bilateral anterior subcapsular cataracts. No other neurologic abnormalities have been previously reported in affected individuals. It is, however, of interest that 1 of Reyersbach's patients had 1 sibling with spastic paraplegia and 2 were mentally retarded. None of these individuals had renal lesions, however. Among the most important studies of this syndrome are the works of Perkoff and associates,[1,2] Reyersbach and Butler,[4,7] and Goldbloom and associates.[8]

CASE REPORTS

Case 1. A 22-year-old white Canadian male, oldest of 3 siblings, was admitted because of impending kidney failure. He was known to have proteinuria and microscopic hematuria since the age of 1½ years and had been hospitalized elsewhere for kidney trouble at irregular intervals. At 11 years of age, a moderate degree of bilateral nerve deafness was recognized and audiometrically documented. At age 18, he was noted to be relatively asymptomatic, except for the hearing loss and recurrent upper respiratory infections. At that time, a blood pressure of 140/100 was noted and unusual bilateral anterior subcapsular cataracts were also seen. Proteinuria, microscopic hematuria, pyuria, and a few casts were present. The urea clearance was reduced to 43% of normal and the clearance of creatine was slightly abnormal.

In 1957, the patient began to complain of intermittent drowsiness and polyuria as well as nausea. Since that time, he has had progressive kidney failure.

Family history revealed that a younger brother died at the age of 5 years following the sudden

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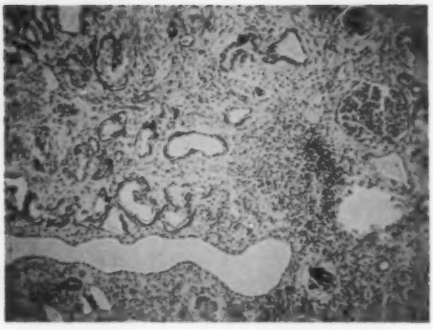

Fig. 1. Microscopic appearance of the kidney lesion in Case 2. Interstitial mononuclear infiltration; tubular atrophy with frequent secondary calcification; relatively intact glomeruli. Hematoxylin-eosin stain

onset of seizures and coma. Autopsy revealed bilateral subdural hematomas and bilateral contracted kidneys. Histologically, the kidneys showed an interstitial nephritis. Another sibling died at age 10 of uremia. Autopsy findings revealed a peculiar form of interstitial nephritis. The mother of the patient had hypertension and signs of renal disease. These cases have been discussed in detail in a previous publication by Goldbloom and associates.[5] In their paper, our patient was case 4.

The patient's first neurologic examination was in February 1958. At that time, he specifically denied any symptoms suggestive of peripheral neuropathy. His mental performance was slightly under average on most tests. No focal neurologic abnormalities were found except for the bilateral decreased hearing. Deep tendon reflexes were normally present throughout. There was no sensory loss. The audiograms showed a 30 to 40 decibel hearing loss at 125 to 500 cps, increasing to a 50 decibel loss between 2,000 to 8,000 cps.

One month later, the patient was readmitted because of continued kidney failure and a tendency toward carpopedal spasms. He was weak and shaky, almost unable to support his own weight. He had a definite loss of memory for recent events and difficulty in concentrating. His orientation was still intact. The eyes were unchanged, and no new findings could be seen in the cranial nerve examination. There was slight tremor distally in both hands with flapping characteristics. There was a moderate and generalized weakness, more pronounced in the distal portions of the extremities, particularly the legs. Deep tendon reflexes were absent at the knees and ankles at a time when they were clearly present in the upper extremities. Plantar responses were flexor. Vibration sense was absent in the toes and somewhat diminished in the ankles. Position sense was slightly abnormal in the toes. There was a distal superficial sensory loss to pin, cold, and touch. At this time, serum potassium was 6.7 mEq. per liter. Despite dialysis, his neurologic abnormalities were not modified and did not change subsequently. He died two months later after an unsuccessful attempt had been made to perform a homologous kidney transplant.

At autopsy, both of the patient's own kidneys were atrophic with nodular elevations of the surface due to dilated tubules and associated fibrosis There was marked atrophy of tubules with extensive parenchymal fibrosis. The glomerular changes were less severe in comparison to the tubules. There was histologic evidence of homograft rejection of transplanted kidneys.

The nervous system examination was restricted to the central part. No abnormalities were found in the brain, cerebellum, brain stem, or spinal cord. A small piece of a proximal segment of a pelvic nerve taken from the area of the lumbosacral plexus failed to show any abnormality.

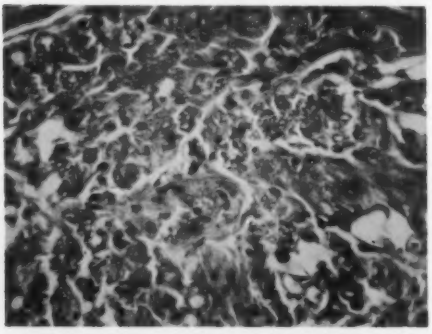

Fig. 2. Cross section of nerve. Relative increase of interstitial and endoneurial collagen. Van Gieson's stain

Case 2. Our second case is a 22-year-old white male with a family history of renal disease in 2 brothers. Hematuria was first noted at the age of 5 years. Later, recurrent hematuria, albuminuria, and pyuria were found. At about the same age, it was noted that the patient was bilaterally deaf, a disability which was clearly progressive.

Five months prior to his last admission, progressive signs of renal failure developed, with hypertension, severe anemia, and an elevated nonprotein nitrogen (at least twice as great as 300 mg. per cent). Three months prior to admission the patient noticed the onset of sensations of pins and needles in both feet associated with progressive weakness in both lower extremities and unaccompanied by edema.

On admission, the neurologic examination revealed a lethargic, pale, well-oriented individual who was bilaterally deaf. Distal weakness in both

lower extremities was marked and was associated with the absence of deep tendon reflexes at the knees and ankles. There was diffuse distal sensory loss to pin prick, touch, and vibration and position sense in both lower extremities. The rest of the neurologic examination was normal.

Subsequent examination of the nervous system in the following two months of hospitalization showed marked progression of his symptomatology. He became progressively weaker until he coundn't ambulate. There was weakness in dorsiflexion, eversion and inversion of both feet, and moderate weakness of the quadriceps, greater on the left than on the right. Paresthesias had increased, and the sensory examination revealed decreased pin and touch sensation up to the level of the lower thighs. The patient's initial serum potassium was under 5, later rising to 6 and 7 mEq. per liter.

Audiograms revealed a 50 to 60 decibel hearing loss up to about 2,000 cps and then a 90 decibel hearing loss to 3,000 cps.

The patient was dialyzed, and a kidney transplant was attempted after total body irradiation of 200 r. The transplanted kidney failed to function and patient expired twenty days later.

Postmortem changes revealed contracted granular kidneys, with superimposed acute pyelonephritis and hydroureter on the right. Microscopic changes showed a chronic interstitial pyelonephritis, involving mainly the tubules with secondary glomerular involvement. There was extensive calcification of tubules and focal calcification of soft tissue (Fig. 1).

The macroscopic examination of the peripheral nerves from the upper and lower extremities did not show any abnormalities. The nerves were thin and showed no segments of hypertrophy of localized abnormalities. No unusual collagen adherence was noted, and the dissection of the nerve bundles was easy.

The specimens were fixed in formalin and subsequently embedded in celloidin or paraffin or studied in frozen sections.

With routine staining technics of hematoxylin and eosin, the general nerve architecture was disorganized, and, although many nerve fibers appeared normal, the myelin sheaths of many had disappeared. Loose collagen tissue was present in the degenerated areas. In most areas, the endoneurium was normal. Occasionally, however, the endoneurial collagen appeared thicker than normal (Fig. 2). The Schwann cells, though normal, were diminished in number. An increased amount of fibroblastic nuclei could be readily seen. No acute or chronic inflammatory cell reaction was noted in the nerves, in the adjacent collagen, or around the blood vessels. A large number of macrophages were seen inside altered nerve fibers and in between some well-preserved fibers (Fig. 3). Most of the macrophages were filled with granulous material and some of them resembled foamy cells (Fig. 4). The general distortion of the architecture and the macrophagic reaction was confirmed

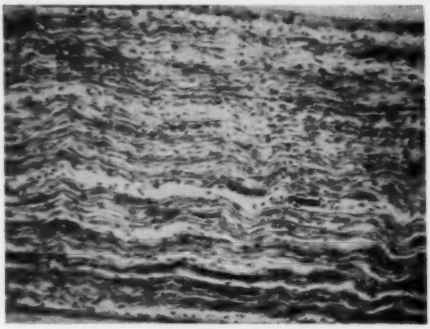

Fig. 3. Longitudinal section of nerve. Distortion of general nerve architecture; no acute inflammatory reaction; great abundance of macrophages. Nissl stain

in frozen sections stained with cresyl violet. No unusual metachromasia was noted.

Sections stained with Luxol fast blue, Weigert, Loyez, Mahon, or Spielmeyer technics showed a widespread demyelination (Fig. 5). The alteration, though diffuse, tended to be more pronounced in the distal portion of long nerves. A few nerve fibers were normally preserved, but the majority of the myelinated fibers stained poorly with an unusually loose network appearance. Fragmentation of myelin and globule formation were often seen. Dilantin of myelin sheaths was frequently noted. In longitudinal sections, dilation and vesicular formation and powder-like destruction of the myelin sheaths were seen to occur in segments. Most dilated myelin tubes appeared empty, but rarely a thin black impregnated axis

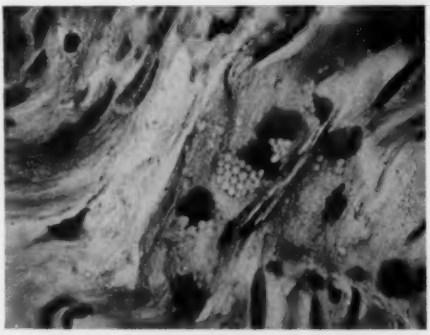

Fig. 4. High power magnification of longitudinal section of nerve. "Foamy cells," fat-loaded macrophages. Hematoxylin-eosin stain

cylinder was still present and eccentrically placed, as if somehow displaced by an unstained material (Fig. 6). No clear-cut segmental degeneration of the myelin as a pattern was noted.

Large amounts of neutral fat could be seen in Oil Red O or Scharlach R stains. Few droplets of neutral fat were seen free in the tissue. Most frequently, they were found in the cytoplasm of macrophages.

The examination of frozen sections under polarized light shows that, besides neutral fat, many cholesterol crystals were seen in the cytoplasm of macrophages or free in the tissue.

In some sections, particularly from distal portions of nerves of the lower extremities, the neutral fat stains produced a light orange-brown color. Close examination under polarized light revealed mainly cholesterol crystals with lesser amounts of less optically oriented substances, probably sphingolipids (Figs. 7 and 8).

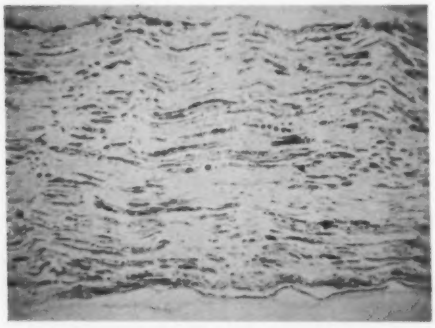

Fig. 5. Longitudinal section of nerve. Widespread demyelination. Weigert stain

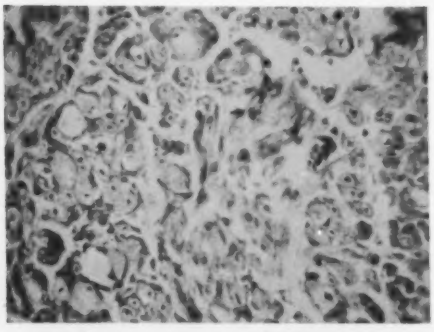

Fig. 6. High power magnification of cross section of nerve. Dilation of myelin tubes with secondary dilation. Eccentric axis cylinders. Masson trichrome-Bodian stain

In silver stains, there were many more axis cylinders than one would expect. This was particularly true of small nerve bundles. The total number of preserved axis cylinders were diminished.

Whereas small-sized nerve fibers seemed to be more frequently spared, large ones were frequently absent or severely affected.

Although occasionally the endothelial cells of small blood vessels and capillaries showed some degree of swelling, there was no clear-cut abnormality of blood vessel walls and no transudate or exudate could be visualized.

No PAS + material outside of the myelin could be seen. Alcian blue stained material was noted in between the nerve fascicles and occasionally inside myelin sheaths (Figs. 9 and 10). In the interstitial space, this material could be distinguished from the endoneural collagen. Here, the Alcian blue positive material usually stained as a loose irregular striae rather than as a homogenous material. This could also be seen in frozen sections counterstained with neutral red.

The proximal portions of the nerves and the anterior and posterior roots were much less involved. The roots of upper thoracic and cervical segments of the spinal cord were normal.

The spinal ganglia appeared normal with only an equivocal increase of amphocytic glial elements in a few areas.

The spinal cord showed a normal pattern of myelination. There were no alterations of the dorsal columns. Chromatolysis of some neurons of the anterior horns of the lumbosacral segments was seen in cresyl violet stains (Fig. 11).

DISCUSSION

In this case, there is little doubt as to the selective involvement of the peripheral nerve system. Although the neuropathology is rather diffuse, the changes were more marked in the distal portion of the nerves and particularly in the lower extremities. This would explain the failure to find any alterations in the one section of proximal nerve examined in our first case.

The characteristics of the lesions suggest an involvement of the myelin sheaths which dilate abnormally and subsequently disintegrate, giving rise to end products of neutral fats, cholesterol crystals, and lesser amounts of sphingolipids. No inflammatory cellular reaction was found.

The over-all speed of the process did not seem precipitous. The clinical picture evolved slowly over the course of months. Most of the myelin end products are in macrophages and very little is left free in the tissue.

The total picture was compatible with a degenerative or toxic process. In the case of

Fig. 7. Longitudinal section of nerves. Fat loaded macrophages. Oil-Red-O stain

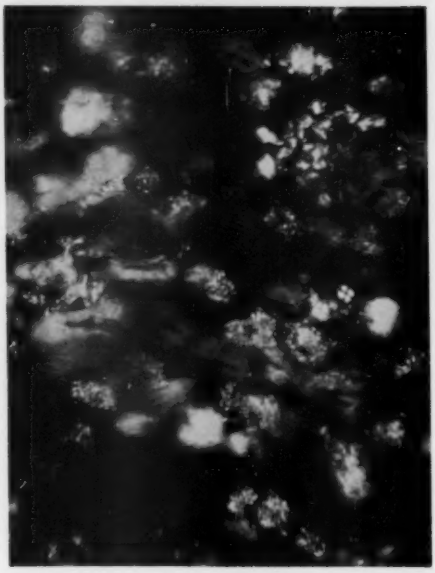

Fig. 8. Same as Fig. 7. Polarized light appearance

the nervous system, it is difficult to be sure whether there is any particular meaning to the large number of fat-filled macrophages in the absence of any other reaction. Perkoff and collaborators,[1] in the study of the kidney lesions, found unusual foamy appearing cells which they interpreted as fat-filled macrophages. These authors advanced the hypothesis that the total inherited disorder could be related to a lipid metabolic defect. Suggestions such as these can neither be proved nor disproved with our material. However, it is interesting to speculate whether the kidney "foamy cells" and our lipid laden macrophages are the same.

Krücke[9,10] has suggested that abnormal interstitial fluid, arising as a result of dyshoric vascular abnormalities, may perhaps serve as a precursor to subsequent deteriorations of the nerve fibers. He uses this as an explanation of the pathogenesis of some degenerative neuropathies, specifically some of the interstitial hypertrophic neuropathies.

One might also speculate that the dilation of myelin sheaths that seems to precede their destruction and the existence of some increased Alcian blue deposits in the interstitial tissue argue in favor of a disorder of vascular permeability or abnormal fluid accumulation. The relatively unremarkable amount of acid mucopolysaccharides found, but particularly the scanty information of the normal presence and quantities of materials with these staining qualities, prevents us from drawing definitive conclusions.

The relationship of the neuropathy found in our case with that of other hereditary neuropathies is highly uncertain. The lesion pattern differs widely from that described in Refsum's syndrome. In the latter, or in other cases of hypertrophic interstitial neuropathies, little is known about the processes which lend to the development of the typical onion-shape and hyperplasia of the endoneurium. The histopathologic changes described in such cases are hardly specific and may be the ultimate consequence of various pathogenetic phenomena such as reported in our case.

It should be stressed, however, that the literature is particularly silent on peripheral nerve

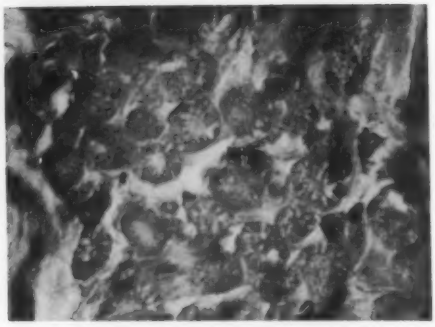

Fig. 9. High power magnification of cross section of nerve. Alcian blue positive material in the interstitial space. Positive PAS myelin sheaths. PAS-Alcian blue stain

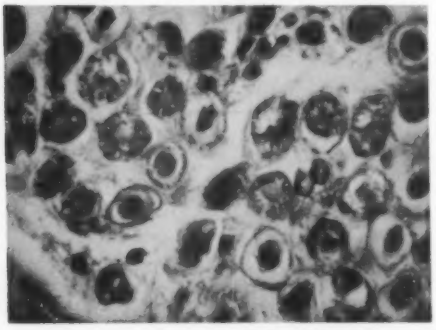

Fig. 10. High power magnification of cross section of nerve. Some Alcian blue positive material in interstitial space. Alcian blue positive material inside myelin tubules. Positive PAS myelin sheaths. PAS-Alcian blue stain

changes or syndromes in the otherwise frequent nonfamilial uremic syndromes.

We have seen occasional peripheral neuropathies similar to the burning feet syndrome in acute cases of uremia and not infrequently evidence of a mild sensory neuropathy in the chronic patients. Severe motor and sensory neuropathies have been rare in our experience of chronic uremia of other causes. We have also seen 5 other examples of a severe progressive sensorimotor neuropathy in chronic uremia in a hospital where uremic patients are frequently seen in terminal stages because of the interest in transplantation and dialysis. The occurrence of sensory loss of this magnitude and the presence of such pronounced weakness has been so unique in our experience, occurring only in these two patients with the same familial disorder, that we do not feel that it is due only to the uremic syndrome. In some way, it must be related to the underlying hereditary disorder.

The variability of the clinical manifestations and the different target organs that can be affected by a single metabolic defect do not need to be emphasized. We would like to suggest that there is probably an inherited abnormality of the peripheral nervous system in both of our patients. The metabolic imbalances and disorders originated by the kidney failure merely act as trigger factors in the production of the clinical and pathologic changes in these patients.

Interpreting the significance of peripheral neuropathy in our cases of hereditary interstitial nephritis is difficult. Only further clinical and pathologic experience will establish whether the neuropathy is genetically determined or is a consequence of the metabolic disorder that accompanies the terminal stages of kidney failure in this disease. Chronic edema does not seem to play an important role in our cases. The first patient was never affected by it.

The second alternative seems to be supported by the fact that in both cases the peripheral symptomatology made its appearance in the last few months of life in the face of irreversible kidney insufficiency.

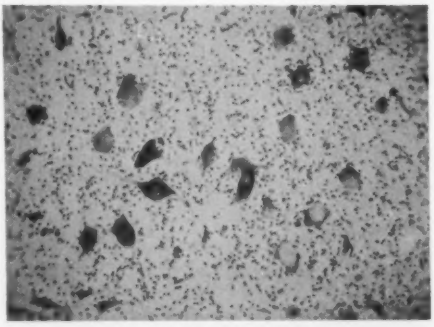

Fig. 11. Anterior horn of upper sacral segment of spinal cord. Chromatolysis of some motor neurons. Cresyl violet stain

SUMMARY

Two cases of hereditary interstitial nephritis and deafness, which had an associated symmetrical progressive peripheral neuropathy, are presented. The pathology of one of the cases is reviewed and an attempt is made to assess the significance of both cases.

Our acknowledgment to Miss Carole Siegel for her secretarial assistance, to Miss Mary McNelly for her technical assistance, and to Mr. Leo Goodmann for his microphotographic work.

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Ability to distinguish two cutaneous stimuli separated by a brief time interval

Joseph B. Green, M.D., Charles L. Reese, M.D., J. J. Pegues, M.D., and Frank A. Elliott, M.D.

RECENT INVESTIGATIONS of cutaneous sensibility have emphasized spatial rather than temporal elements in perception. The ability to perceive two simultaneous but spatially separated stimuli is reported to be impaired in patients with disease of the central nervous system.[1] Defects in perception so identified include extinction, which was defined by Bender as a "process in which a sensation disappears or a stimulus becomes imperceptible when another sensation is evoked by simultaneous stimulation elsewhere in the sensory field."[1]

The discrimination of successive stimuli confined to one area of a sensory field is not often tested. One of the few such measurements is the critical flicker-fusion frequency which reflects the ability to distinguish flashes of light separated by a brief time interval. The critical flicker-fusion frequency is said to be reduced in patients with cerebral lesions.[2]

It was decided to investigate the appreciation of temporally related cutaneous stimuli by a method analogous to the flicker-fusion test of visual perception. A procedure was devised to test the ability to identify cutaneous stimuli separated by a brief time interval. Normal subjects as well as patients with a variety of neurologic lesions were examined.

METHOD

An electronic stimulator (Model 104-A, American Electronic Laboratories) was employed which was capable of delivering pulses of varying duration singly or in pairs. A suction-type precordial electrocardiogram electrode was used for stimulation, with a limb lead as ground. The area of the skin to be tested was moistened with electrode jelly. Single shocks of 0.1-msec. duration were given to establish thresholds for "touch" and at higher voltage for "pain." Painful shocks were then paired, first at several hundred milliseconds apart, and then brought closer together until the two were felt as one (fusion). They were then gradually separated until each was distinctly and separately perceived. The intervals at which fusion and separation occurred were noted. Series of paired shocks were interrupted in random fashion by the interjection of single shocks, both to assess reliability of testimony and to prevent perseveration or conditioning of response.

A variety of cutaneous areas were stimulated in the initial phase of the study, but it was eventually decided to test the skin of the thumb with the ground electrode on the forearm. The duration of individual stimuli was maintained at 0.1 msec. at a voltage which was just painful, for reasons which will be discussed in a later section.

In addition to normal subjects, patients with sensory loss due to a variety of lesions were studied. The emphasis, however, was on the testing of patients who had sensory deficit due to intracranial disease. All subjects were also studied by examination of pinprick, touch, position, and vibratory sensation, stereognosis, graphesthesia, and two-point spatial discrimination.

RESULTS

Normal subjects. *Single shocks.* When a normal subject was given a brief (0.1-msec.)

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shock at threshold voltage, he reported a sensation of a discrete tap or touch directly under the center of the stimulating electrode. With increasing voltage, the tap grew stronger and assumed the quality of a prick. With still higher voltage, the sensation was described as a painful shock. The strength-duration characteristics of threshold stimulation are described in the figure. The curves for stimulation of the left and right thumbs correspond closely. It may also be seen that, at 0.1 msec., the slope of the curve is level and further increases in duration of the stimulus do not greatly alter threshold. For this reason, a stimulus duration of 0.1 msec. was chosen for routine testing.

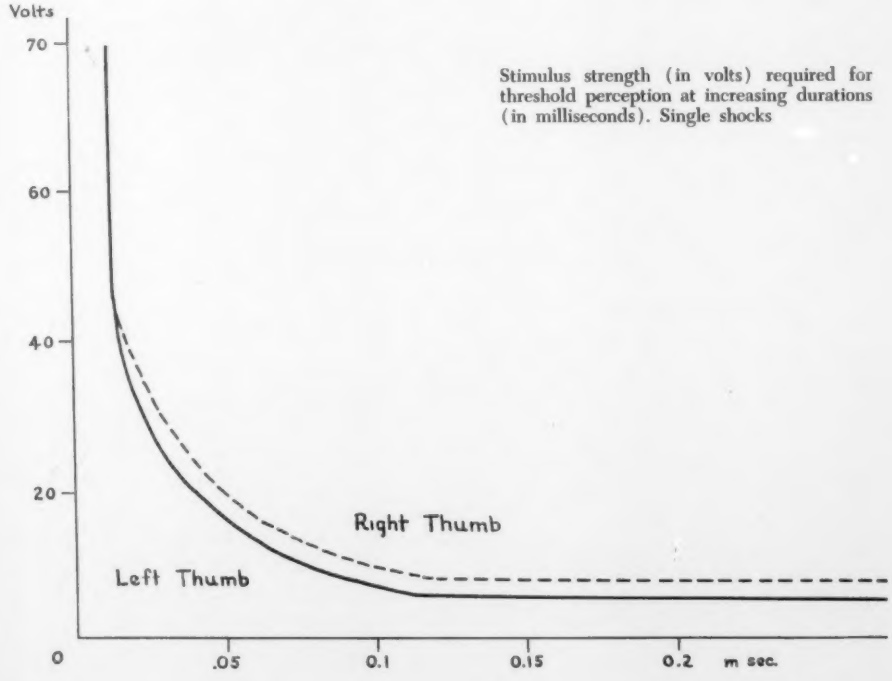
Paired shocks. Normal individuals can feel nonpainful shocks 100 msec. apart as distinct and separate. The fusion time (interval at which 2 shocks, separated by decreasing time interval, are felt as 1) is always less than separation time (interval at which 2 shocks, separated by increasing time interval, are felt as 2).

The lowest fusion and separation times, that is, the most accurate discriminations when related to body surface, were obtained on stimulation of face and thumb. Fusion times were 20 msec. longer on testing the large toe and 40 msec. longer on examination of trunk or abdomen.

When shocks were made strong enough to just produce pain, performance improved and fusion-separation times of 20 to 40 msec. were recorded (face or thumb). Increasing stimulus intensity still further had a deleterious effect on discrimination. To secure the optimum discrimination of paired shocks, stimulus strength was increased in all instances to a painful level before testing was concluded.

A curious phenomenon was noted during the administration of painful paired shocks. When the members of a pair were separated by 100 msec., each was felt to be stronger. This intensification waxed for 30 msec. and disappeared just before fusion.

As the result of a large number of measure-



Stimulus strength (in volts) required for threshold perception at increasing durations (in milliseconds). Single shocks

TABLE 1

COMPARATIVE FREQUENCIES OF VARIOUS TYPES OF SENSORY LOSS IN 23 CASES OF CEREBROVASCULAR DISEASE

Sensory function	*Number of cases in which impaired*
Pinprick	18
Position sense	11
Vibration sense	8
Two-point discrimination	9
Stereognosis	10
Paired shock discrimination	14

TABLE 2

COMPARATIVE FREQUENCIES OF OTHER TYPES OF SENSORY LOSS IN 14 CASES IN WHICH THERE WAS DEFICIENT DISCRIMINATION OF PAIRED SHOCKS

Sensory function	*Number of cases in which impaired*
Pinprick	6
Position sense	8
Vibration sense	6
Two-point discrimination	6
Stereognosis	7

ments in normal individuals, it was concluded that the fusion or separation times of homologous areas of the body never differed by more than 20 msec. Hence, a difference of 50 msec. between thumbs (or face) in respect to either fusion or separation time was considered abnormal.

Patients. Tables 1 and 2 list the frequency of various types of sensory deficit in 23 patients with cerebrovascular lesions. Nearly all had hemiparesis. Aside from differences in pinprick appreciation, impairment of discrimination of 2 stimuli in rapid succession was the most frequent finding. This impairment could not be correlated with any other form of sensory deficit and occurred as the only sensory abnormality in 2 patients with hemiplegia. In several patients, fatigability of discrimination could be demonstrated. This was prominent in several patients with cerebral infarction but was particularly well developed in 2 cases of parietal lobe tumor. Examples of fatigability are seen in the following 3 case histories.

Case 1. J. D., a 50-year-old toll collector, had been admitted with the complaints of indistinct vision for objects to his left, progressive "numbness" and clumsiness of his left hand, and left-sided headache. Neurologic examination revealed left homonymous hemianopsia and increased deep tendon reflexes on the left. Pinprick and vibration sense were defective over the left hand and foot. An electroencephalogram revealed a right parieto-occipital delta wave focus.

In electrical testing of the left thumb, he was able to distinguish 2 shocks 180 msec. apart, but, as testing was continued, he made consistent errors in identifying separate stimuli as much as 700 msec. apart. After two minutes' rest, he could again distinguish 2 shocks separated by 180 msec. Fatigue developed repeatedly during testing sequences. In testing of the right thumb, fusion occurred at 100 msec. No fatigue was demonstrated.

At surgery, a right parietal glioma was partially removed. Histologically, it proved to be an astrocytoma, grade II. Testing was repeated after craniotomy with essentially the same results.

Case 2. J. McG., a 55-year-old retired soccer player, was admitted because of an inability to use his left hand properly for two days. Neurologic examination revealed clumsiness and slight weakness of the left hand. He had decreased appreciation of pinprick on the left side of the body and face. A marked reduction of position and vibration sense was present in the fingers of the left hand. Electrical testing of the left thumb revealed discrimination of paired shocks 200 msec. apart. With continued testing over a period of ten minutes, he failed to distinguish shocks 700 msec. apart. After a rest of one minute, he was again able to discriminate between stimuli 200 msec. apart. Testing of the right thumb revealed fusion at 100 msec. with no fatigue.

Right carotid arteriography revealed a block in the internal carotid artery just above the bifurcation. This was confirmed by surgical exploration. A clot was discovered which extended into the intracranial portion of the vessel. Electrical testing after the operation showed no change from previous findings.

Case 3. F. R., a 45-year-old man, had the chief complaints of progressive left hemiparesis and headache. He also reported "numbness" of the left hand. He had last been well two months before. Neurologic examination revealed left hemiparesis, left central facial weakness, and bilateral papilledema (2 diopters). Pinprick sensation, position, and vibration sense were reduced in the fingers of the left hand. Astereognosis was present. Two-point spatial appreciation was absent in the finger tips of the left hand.

Paired shocks to the left thumb fused at 120 msec. After testing for several minutes, fusion occurred at 300 msec. A short rest restored initial discriminatory ability. Paired shocks to the right thumb fused at 70 msec. with no fatigue. A right carotid arteriogram revealed a tumor stain in the parietal area. At craniotomy, a gliomatous tumor

was found to be present just below the cortex and to overlay the sensory strip anteriorly.

As mentioned, two patients had as the only sensory change a failure to discriminate a sequence of two stimuli. A description of one of these cases follows.

Case 4. C. G., a 55-year-old woman, was noted to have right homonymous hemianopsia and partial visual agnosia. A year previously, she had right peripheral facial weakness, sudden in onset. No objective sensory deficit could be identified on routine testing at that time. Electrical testing revealed a fusion time of 100 msec. for the right thumb, with fatigue to 400 msec. The fusion time for the left thumb was 100 msec., and no fatigue occurred.

Three months later she suddenly became aphasic, and right hemiplegia and hemianestheisa developed. A left carotid arteriogram disclosed several areas of narrowing and plaque formation in the internal carotid artery.

It was noted on occasion that initially negative results did not preclude abnormalities later, especially in progressive lesions. Such a case was that of J.C.

Case 5. J. C., a 45-year-old hypertensive man, awoke with mild right hemiparesis. There were no sensory changes on routine testing. Fusion time was 60 msec., either thumb. Over the next four days, the right hemiparesis worsened markedly. Reexamination after this period showed markedly impaired position sense in the right fingers and toes and decreased vibratory sensation in these parts. Fusion time (right thumb) was 200 msec. Fusion time on stimulation of the left thumb was 70 msec.

Two patients with Gerstmann's syndrome had excellent appreciation of paired shocks. Basic sensory modalities were not disturbed in these patients.

A number of patients (15) with sensory loss due to peripheral nerve or cord lesions have been tested. So far, no instances of impairment in paired shock discrimination have been detected. Diagnoses have included carpal tunnel syndrome, multiple sclerosis, cord compression, and brain stem neoplasm. Commonly, in these cases, there has been an increase in the electrical thresholds for "touch" and "pain." Increased thresholds have similarly been encountered in every patient with sensory loss due to cerebrovascular disease and cerebral neoplasm. However, quantitatively similar differences in thresholds between two sides of the body have occasionally been found in normal subjects. When such differences occurred in right-handed individuals, the left side was invariably more sensitive. This phenomenon is the subject of a current investigation.

DISCUSSION

The subjective responses to graded electrical shocks to the skin observed in this study are similar to those recorded by Landau and Bishop.[3] These investigators noted that stimuli separated by 200 msec. tended to summate. In the present study, it was found that shocks 70 to 100 msec. apart were felt to be stronger. This interval is identical to a phase of sensory facilitation determined by Gastaut and associates[4] for the visual cortex. The latter authors measured the evoked potentials induced in the visual cortex by a second light flash at various intervals after a primary stimulus. They found an absolute refractory period of 20 msec. and a relative refractory period of 40 msec. When the second flash followed the first by 70 to 100 msec., the ratio of the voltages of the second evoked potential to the first evoked potential exceeded unity, that is, facilitation was present.

It is of interest that some of our subjects were able to distinguish 2 shocks 20 msec. apart, which corresponds to the absolute refractory period of cortical sensory neurons as determined by Gastaut and associates.

Discriminatory ability improved as shocks became painful. The explanation for this is not clear but may involve a stimulation of the reticular formation by the nociceptive impulses. Painful stimuli in animals facilitate cortical responses to acoustic and optical stimuli.[5] Moreover, Lindsley[6] was able to record from visual cortex evoked potentials to each of 2 light flashes 50 msec. apart after reticular stimulation, whereas before such stimulation only 1 evoked potential was observed.

In a recent investigation of human visual discrimination, it was found that the critical interval between 2 flashes of light which permitted perception of both was 73 msec.[6] This value is quite comparable to the minimum interval necessary for the appreciation of 2 nonpainful cutaneous shocks but is much greater than that required for painful impulses. It ap-

pears probable that the latter may by a process of reticular facilitation aid in the cortical resolution of paired stimuli.

It should be stressed that, in this series, impairment of the discrimination between 1 and 2 stimuli was evident only in cases of intracranial disease, either cerebrovascular or neoplastic. Experimental studies of the response to flicker frequencies in animals are pertinent to this observation. Flash frequencies to above 100 per second could be followed by all subcortical stations, but the cortex responded to rates only up to 40 or 50 per second, a value which corresponds closely to the critical flicker-fusion frequency of normal human subjects.[6] Hence, the limiting factor in the temporal separation of light flashes may be the cerebral cortex. Our findings suggest that the appreciation of 2 cutaneous stimuli separated by a brief time interval is likewise a cortical function, a fact consistent with the neurophysiologic studies of the visual system cited. The presence of specific fatigue recorded in many of our cases accords with this concept.

The fact that impaired discrimination between 1 and 2 brief cutaneous stimuli was the only type of sensory deficit specific to intracranial disease would seem to have some practical value. It occurred with or without other forms of sensory loss detectable by conventional methods of testing.

SUMMARY AND CONCLUSIONS

1. A method for testing the appreciation of cutaneous stimuli separated by a brief time interval has been devised.

2. This discriminatory function was found to be impaired in patients with cerebrovascular disease and parietal lobe tumors.

3. Thus far, lesions of the spinal cord or peripheral nerve have not been associated with deficient identification of paired stimuli.

4. Neurophysiologic correlates of these clinical observations are discussed.

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Effect of serotonin on behavior, electrical activity of the brain, and seizure threshold of the newly hatched chick

With a note on the production of seizures presenting 14-per-second and 6-per-second positive spikes

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SINCE THE RECENT DISCOVERY of relatively large amounts of naturally occurring serotonin in the brain,[1,2] much attention has been directed toward the neuropharmacologic action of this substance. Evidence has been gathered indicating that serotonin is a possible neurotransmitter within the central nervous system;[3] other investigators have attributed a synaptic inhibitory function to serotonin.[4] A problem common to these investigations employing a parenteral route of administration, however, is that serotonin crosses the blood-brain barrier with difficulty. Costa and Aprison[5] have demonstrated that only 1% of injected serotonin is deposited in the brain. Bogdanski, Weissbach, and Udenfriend[6] make the statement that "central pharmacological effects of serotonin are difficult to evaluate because the quantities which cross the blood brain barrier after parenteral administration are too small to permit chemical detection." With these data in mind, the present investigation was undertaken to determine the effects of various dosages of serotonin on external behavior, electrical activity of the central nervous system, and seizure threshold of an animal whose blood-brain barrier has not yet fully developed. Recalling the fact that the blood-brain barrier of the newborn is permeable to substances unable to penetrate the adult barrier, as evidenced by the disease kernicterus, the newly hatched chick was selected as the experimental animal. Studies of the blood-brain barrier of the embryonic and newly hatched chick using radioactive chloride and thiocyanate[7] have indicated that this animal does not develop a mature blood-brain barrier until three to four weeks after hatching. By using the chick, it was hoped that the neuropharmacologic action of serotonin could be more closely evaluated by achieving higher brain levels of serotonin than those previously attained in mature animals.

MATERIALS AND METHODS

About 300 New Hampshire chicks between the ages of 2 and 6 days after hatching were used in this study. The creatinine sulfate salt of serotonin (5-hydroxytryptamine) dissolved in normal saline to a concentration of 10 mg. per cubic centimeter was injected. Although the femoral vein was cannulated and the drug injected intravenously in a number of animals, the more convenient intraperitoneal route was used routinely. With the majority of the chicks weighing between 30 and 45 gm., 2 dosage ranges of serotonin were used, 0.01 to 0.05 mg. per gram of body weight constituting the low range and 0.06 to 0.10 mg. per gram of body weight making up the high range. Modifications of external behavior and electroencephalographic recordings were noted after injection.

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To test for serotonin-induced alterations of seizure threshold, the animals were subjected to an intraperitoneal injection of 6 mg. of Metrazol or a maximal (tonic-clonic) electroshock. A statistical analysis of the results of seizure inhibition in treated animals as compared to control animals was made using the chi-square method.

Electrical recordings were made with a Grass model III D 4-channel electroencephalograph. The unanesthetized chick was mechanically restrained by means of a small harness. The skull was exposed and stainless steel needle electrodes were inserted through the skull to a depth of 1 mm. over the right and left cerebral lobes. Monopolar recordings were taken employing the comb as the common reference point because of its location and minute electrical activity.[8] Control animals were injected with comparable amounts of normal saline and subjected to the same experimental procedures.

OBSERVATIONS

External behavior. After administration of serotonin in dosages from 0.01 to 0.05 mg. per gram, the chick becomes ataxic with incoordinate placement of limbs. It assumes a squatting position and lapses into a stuporous condition. Decreased muscle tone is evidenced by

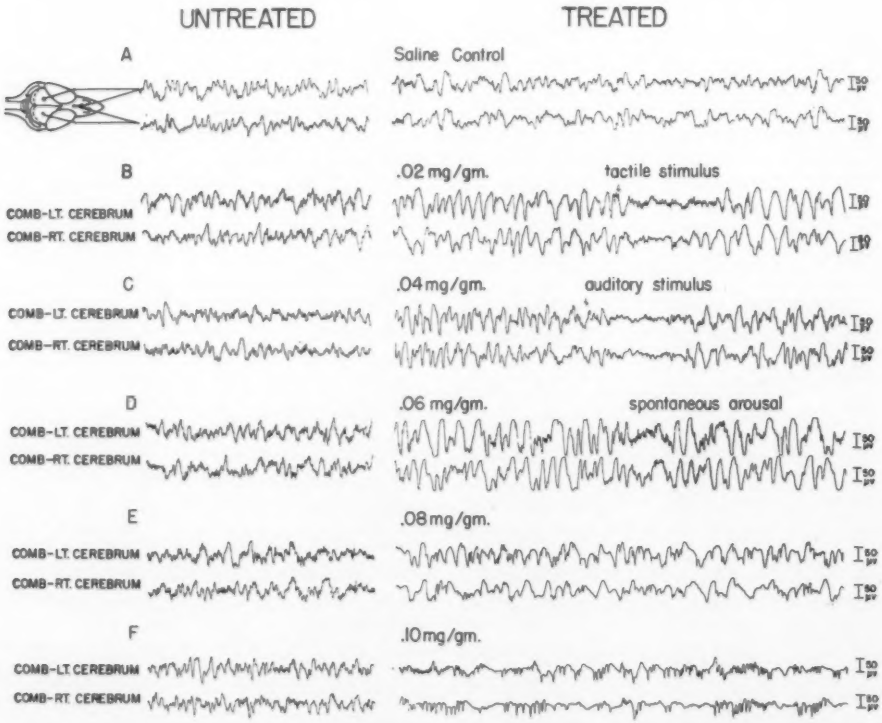

Fig. 1. Selected records illustrating electrical activity of cerebral lobes before and after treatment with serotonin at various dosages. Lower range doses B and C produce increase in amplitude of slow waves with diminution of superimposed low-voltage, fast activity seen in normal tracing [A]. Note arousal response to auditory and tactile stimuli in B and C. Similar response in D is spontaneous. Positive spike potentials appear in recordings after injection of higher doses of serotonin [E and F].

drooping wings. Auditory and tactile stimuli evoke an immediate arousal reaction in which the head is raised and the eyes opened. When the stimulus is discontinued, the chick relapses into the stupor. Body movements and changes of position are infrequent, and the chick can be induced to walk only with difficulty. Equilibratory reflexes remain intact, the chick righting itself if suddenly placed on its back. However, if restrained in this or other abnormal positions until the stuporous condition returns, the chick maintains the artificially induced posture until rearoused by another stimulus, at which time it rights itself.

As the dose is increased to the higher range of 0.06 to 0.10 mg. per gram, the chick manifests equilibrium responses but with difficulty because of the increased muscle flaccidity. Spontaneous arousals from the stupor are more frequent. Many of the animals injected with the 0.10 mg. per gram dose exhibit a clonic convulsion characterized by bilateral extensor spasms of the extremities. The convulsion is usually followed by death.

Electrical activity. The fundamental rhythmic frequency of the resting electroencephalogram of the newly hatched chick ranges between 2 and 6 high-amplitude waves per second. Superimposed on the dominant rhythm is a low-voltage, fast-frequency background discharging at a rate of 35 to 45 per second.

Intraperitoneal injection of serotonin in the lower dose range (0.01 to 0.05 mg. per gram) produced an increase in the amplitude of the slow rhythm with a diminution to complete elimination of the fast-frequency background activity. Although the shape of the slow wave appears simplified, the frequency remains unaltered. Tactile and auditory stimuli evoke a brief run of low-voltage, fast-frequency activity corresponding to a brief period of arousal (Fig. 1).

Increasing the dose of serotonin to near convulsant levels (0.08 mg. per gram) results in less effective elimination of background activity and increasing numbers of positive spike potentials, that is, those without an upward deflection or negative phase, appearing in the background discharge. At or immediately below convulsant doses, the positive spike potentials appear to the exclusion of the dominant rhythm (Fig. 1). The average frequency of the positive spike potentials is between 12 and 16 per second, with the extremes varying between an occasional spike and bursts of 24 per second. The spikes are not necessarily recorded synchronously in both channels and are not associated with visible external movements. At convulsive dosage, the positive spikes usually precede but often are interposed with the electrical manifestations of the clonic spasms. Electrical quiescence follows the convulsion. When the animal survives the serotonin-induced seizure, a gradual return of normal electrical activity is obtained.

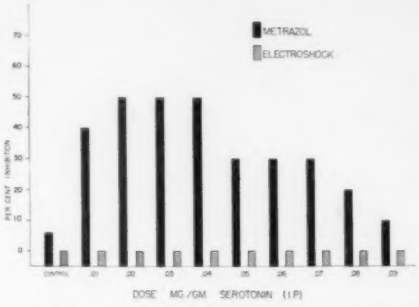

Fig. 2. Graph summarizing effectiveness of anticonvulsant activity of various dosages of serotonin. Each bar indicates per cent protection against Metrazol and electroshock seizures afforded by serotonin in series involving 10 or more animals at each dose.

Anticonvulsant property. The effects of various doses of serotonin on seizure threshold are summarized in Figure 2. Lower doses (0.01 to 0.05 mg. per gram) are successful in producing a significant increase of threshold to a Metrazol-induced seizure. This fact is supported by a statistical evaluation of the data using the chi-square method. The number of Metrazol seizures inhibited by higher doses (0.06 to 0.09 mg. per gram) is not statistically significant. Serotonin does not modify the tonic-clonic manifestations of a Metrazol seizure when it does occur, although, in some animals, it is successful in delaying onset of convulsive activity as long as two hours after Metrazol has been injected. Serotonin in the dosages employed is completely ineffective in either preventing or modifying electroshock seizures.

DISCUSSION

The reticular activating system, located in an area of the brain rich in serotonin,[1] plays an important role in exerting a pronounced facilitatory influence on spinal motor activity as well as maintaining the state of wakefulness with its characteristic low-voltage, fast-frequency electroencephalogram.[9] Lesions placed in the rostral end of the reticular formation of the cat produced a syndrome, resembling catalepsy in man, characterized by somnolence, pronounced lack of motor initiative, marked disinclination to walk, and acceptance of passively induced poses.[10] It was further noted by Magoun[9] that it is possible to induce in animals with lesions in this area a degree of behavioral arousal and to activate the electroencephalogram by auditory and somatic stimulation, with the arousal terminating almost immediately upon cessation of the stimulus. From our results, it is apparent that serotonin in lower dosages has a central depressant effect. The similarity between the studies noted above and the syndrome observed in this experiment suggests the reticular activating system as the site of serotonin's depressant action. Further support for this conclusion is provided by Vogt, Gunn, and Sawyer,[11] who observed marked lowering of the electrical activity of the midbrain reticular formation after intraventricular injection of serotonin in the cat.

There is a possibility that serotonin acts peripherally, inhibiting sensory reception with a secondary depression of the reticular formation. However, the response of the animal to tactile and auditory stimuli indicates functional receptor organs. The arousal reaction observed in an animal with a nonfunctioning reticular formation results from the direct arrival of afferent impulses at a point rostral to the reticular activating system via the long sensory paths.[9]

Chemical mediators of nerve transmission are considered to work through membrane depolarization of nerve cells. If an excess of the mediator is present, temporarily overcoming the ability of the specific enzyme to metabolize the mediator, a volley of postsynaptic impulses may be produced. The continued presence of large excesses of the mediator results in persistent depolarization and cessation of function of that part of the nervous system. If the evidence for the central neurotransmitter function of serotonin is accepted,[3] the depression of the reticular activating system may be the result of the presence of excess serotonin causing the persistent depolarization of this system.

Injection of the higher doses of serotonin into the chick resulted in the recording of positive spike potentials with an appearance and frequency similar to the 14- and 6-per-second positive spike potentials described by Gibbs and Gibbs[12] in their study of the "sleep" electroencephalogram of epileptics. Since the symptomatology of cases exhibiting this type of discharge indicated a thalamic or hypothalamic lesion and the polarity of the spikes suggested a "disordered energy release" from a subcortical site, they advanced the 14- and 6-per-second positive spikes as electroencephalographic evidence of hypothalamic and thalamic epilepsy. The resemblance between the positive potentials recorded in this experiment and those of Gibbs and Gibbs indicates the hypothalamus or thalamus as the possible site of origin of the experimentally produced spikes.

Since monoamine oxidase, the enzyme which destroys serotonin, is normally concentrated in the hypothalamus,[13] higher brain levels of serotonin than those sufficient to depress the reticular formation may be required to overcome the catabolic ability of the enzyme in the hypothalamus. Thus, it has been suggested that temporary flooding of the synapses in this region with high doses of serotonin results in the production of volleys of postsynaptic impulses which are recorded electrically as the positive spike potentials.

The criticism that the positive spike potentials recorded after injection of serotonin are actually artifacts of technic or muscle potentials emanating from the area of the reference electrode has been advanced. However, the failure to record this type of discharge from control animals and the fact the spikes do not necessarily appear synchronously in both channels argue strongly against these objections.

Although the 14-per-second and 6-per-second positive spikes and the commonly associated vegetative symptomatology are believed to be indications of dysfunction of the diencephalic

nuclei, and more specifically the central autonomic nervous system, no experimental evidence to support this possibility has been forthcoming.[14] The production of this type of spike potential by the parenteral administration of a naturally occurring neuropharmacologic agent, which is normally concentrated in the diencephalon and especially in the hypothalamus[13] and which has been implicated as the neurotransmitter in the central parasympathetic nervous system,[1] may provide the supportive evidence which hitherto has been lacking. Although it would be impossible on the basis of our results to propose an abnormality in serotonin metabolism as the pathophysiologic basis of this electroclinical syndrome, this point warrants further investigation.

Sudden and temporary and then persistent depolarization of the diencephalic nuclei and other portions of the central nervous system may account for the clonic convulsions and subsequent electrical quiescence produced by convulsant doses of serotonin. Peters, Vonderahe, and Powers[15] suggest that the striatum, diencephalon, or both are responsible for clonus.

Peripheral afferent stimuli induce the wakeful state through excitation of the reticular activating system,[16] and depression of this system results in fewer sensory impulses reaching the higher brain centers. Since sensory and supranuclear mechanisms augmenting and facilitating the direct action of Metrazol on motor neurons are necessary for the convulsant action of this drug,[17] the lethargic response of serotonin-treated animals to Metrazol supports the conclusion that serotonin exerts its depressant effect on the reticular activating system. Conversely, depression of this system offers an explanation for the elevation of seizure threshold after the lower doses of serotonin. The last mentioned point agrees with the statement of Hankoff and associates[18] that depression of the mesodiencephalic activating system raises seizure threshold. Direct excitation of the higher centers, as evidenced by the positive spike potentials and more frequent spontaneous arousal reactions, would account for the relative ineffectiveness of higher doses of serotonin to inhibit Metrazol-induced seizures.

Bonnycastle, Paasonen, and Giarman[19] noted a significant increase in brain levels of serotonin after administration of diphenylhydantoin (Dilantin). The present investigation indicates that the increased levels of serotonin are not responsible for the mechanism of action of diphenylhydantoin, since this drug is one of the most potent of the antiepileptic drugs in its ability to modify maximal electroshock seizures in animals and man. Prockop, Shore, and Brodie[20] noted, however, that manoamine oxidase inhibitors, which increase brain stores of serotonin, protect against maximal electroshock seizures in rats.

Serotonin has a strong direct vasoconstrictor activity[2] resulting in poor and irregular absorption from the abdominal cavity after intraperitoneal injection. Evidence for this fact was obtained when doses 100 to 150 times smaller than the intraperitoneal doses produced similar electroencephalographic changes in the chick when injected intravenously.

SUMMARY

1. Because parenterally injected serotonin crosses the blood-brain barrier only in minute amounts, the newly hatched chick with an incompletely developed blood-brain barrier was selected as the experimental animal. The effects of various dosages of serotonin on external behavior, electrical activity of the nervous system, and seizure threshold were tested.

2. Serotonin, injected intraperitoneally in the low dosages of this experiment, produced a syndrome characterized by ataxia, decreased muscle tone and motor activity, and stupor. The electrical activity at this time shows an increase in the amplitude of the slow waves with a diminution to complete elimination of the low-voltage, fast-frequency background activity. Auditory and tactile stimuli evoke an immediate arousal reaction. Depression of the reticular activating system is advanced as the basis for these effects.

3. After injection of higher doses of serotonin, positive spike potentials, similar in appearance to the 14-per-second and 6-per-second positive spike potentials advanced by Gibbs and Gibbs as electroencephalographic evidence of autonomic epilepsy, were recorded. In sufficiently high doses, serotinin causes a clonic convulsion which is usually followed by death.

4. Serotonin in the lower dosages produced a significant increase in threshold to metrazol-induced seizures. In doses used in this experiment, serotonin is completely ineffective in preventing or modifying electroshock seizures. Possible explanations for these results are discussed.

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Delayed focal convulsive seizures after head injury in infants and children

A syndrome that may mimic extradural hematoma

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THE CLASSIC CLINICAL HISTORY of extradural hematoma after head injury—transient unconsciousness followed by a lucid interval and then coma—has received great emphasis in medical teaching literature.

It is widely assumed that this sequence invariably means that the patient has a massive hematoma requiring immediate surgical treatment.

However, in infants and children, a similar syndrome which is not due to hematoma is seen after head injury. The clinical sequence is head injury with or without transient unconsciousness, a lucid interval, and then a convulsive seizure leading to coma. The initial seizure is frequently not seen, and the patient is found in postictal coma. Subsequent seizures may not be recognized or, if recognized, may not be treated because the patient is then en route to a neurosurgeon or because the seizure is assumed to be due to intracranial hematoma and the attending physician is reluctant to give depressant anticonvulsant medication. Unless the true nature of the disturbance is recognized and controlled, serious or even fatal complications may result. If the syndrome is recognized and treated, the patient should recover without residual disability.

During a period of one month, 4 children with this syndrome were admitted to the Head Injury Service of the Nemazee and Saadi Hospitals, together with 1 child with classic extradural hematoma treated surgically. This group of cases illustrates the problem of differential diagnosis and management of the 2 lesions.

Case 1. "Postcontusion seizure syndrome." N.A., a 2-year-old girl, was admitted to Saadi Hospital September 21, 1960, after having fallen 8 ft. two hours previously, landing on her head. She was briefly unconscious and then appeared normal but complained of headache. Approximately fifteen minutes after the fall, the child had a convulsive seizure involving the right extremities, lasting for about "half an hour." The patient reached the hospital two hours after injury in deep, flaccid coma. The pupils were dilated but equal. Roentgenograms showed a linear fracture of the left frontal bone. Fifteen minutes after admission, another seizure involving the right extremities occurred. Surgery was alerted, blood was drawn for crossmatching, and intravenous fluids were started. No further seizures occurred. Under continuous observation, the child's neurologic state gradually and progressively improved. The following morning, she was fully active and appeared normal. There was no history of previous seizures in the patient or in other members of the family.

Comment. This child had a recognized focal seizure occurring after a short "lucid" interval. It is unlikely that an extradural hematoma would have reached sufficient size in a period of fifteen minutes to have been responsible for the convulsive seizure. However, it was possible that the seizure resulted from cortical contusion and that an extradural hematoma was developing simultaneously. Her progressive improvement after cessation of the seizure resolved this question.

Case 2. Extradural hematoma. B.G., a 5-year-old boy, was admitted to Saadi Hospital October 8, 1960, after having been injured in

From the Head Injury Service of Nemazee and Saadi Hospitals, Shiraz, Iran.

a fall from his bicycle twenty-four hours previously. He was not unconscious but had a 2-cm. laceration of the left frontal scalp which was sutured at the hospital. The patient appeared otherwise normal and was discharged. Approximately five hours after the injury, the child was irritable, complained of headache, and vomited several times. The following morning, he was lethargic and irritable and was taken to the hospital. No neurologic abnormality was noted by the examiner, and the child was sent home. During the day, he showed increasing lethargy and was again brought to the hospital, at 8:30 P.M. Roentgenograms of the skull at this time were reported to show no fracture. The child was then seen for the first time by the Head Injury Service. At 9:30 P.M., the left pupil was slightly larger than the right. The patient was stuporous but could be aroused by painful stimulation. At 10 P.M., he was comatose; dilation of the pupil had increased, accompanied by ptosis; and progressive paresis of the right arm was noted. The patient was taken to surgery, and a left subtemporal craniectomy revealed a massive extradural hematoma filling the temporal fossa and extending over the convexity of the hemisphere to a depth of 3 to 5 cm. Fracture of the temporal bone was present. The patient's condition improved immediately after operation except for persistence of the pupillary dilation and ptosis. The third nerve paresis had cleared completely, and the patient was entirely well three weeks after operation.

Comment. The fact that a surgical complication was developing should have been recognized earlier, since the child's level of responsiveness deteriorated progressively for at least twelve hours before he showed localizing neurologic changes. In contrast to the cases of cerebral contusion, this patient showed a continuous downhill course, starting a few hours after injury and progressing until relieved by operation.

Case 3. "Postcontusion seizure syndrome." F.S., a 4½-year-old girl, was admitted to the Nemazee Hospital October 21, 1960, after having fallen 4 ft. from a stairway, landing on her head, three hours before admission. She was not unconscious. Approximately one hour after injury, she complained of headache, appeared lethargic, and vomited. She was seen by a local physician who advised hospitalization. On arrival at the hospital three hours after injury, the child was unconscious, with the head and eyes deviated to the left. Abrasion and ecchymosis were seen over the right frontal scalp. Before further neurologic evaluation could be made, a convulsive seizure occurred involving the left extremities and face. Following this, the child remained deeply comatose, responding only to painful stimulation and exhibiting left hemiparesis. Skull roentgenograms did not show fracture. Surgery was alerted, blood was drawn for crossmatching, and intravenous fluid was started. Forty minutes after admission, the patient began a restless thrashing with the right extremities. In anticipation of another seizure, 25 mg. of phenobarbital was given slowly intravenously. Thereafter, the child showed gradual but continuous improvement. Within three hours, the hemiplegia was receding, and, the next morning, the child showed no evidence of neurologic disability.

Comment. This child arrived at the hospital in coma three hours after a head injury from which she was not initially unconscious. The parents denied that the patient had had a seizure before entry, but, in view of her course, it is likely that an unrecognized seizure accounted for the state of coma on admission.

Case 4. "Postcontusion seizure syndrome." M.T., a 3½-year-old boy, was admitted to Saadi Hospital on November 2, 1960, after having been struck in the right frontal area by a swinging door twenty hours previously. He was knocked down but was not unconscious. A 2-cm. laceration of the right frontal scalp was sutured at the hospital and the patient sent home. The following morning, the child was noted to be pale, lethargic, and unsteady in gait. Shortly after these changes were noted, he was seen to fall unconscious with jerking of the limbs. On admission to the hospital, the left arm and leg were flaccid and the right arm was moving purposelessly with a flailing motion. A further seizure was anticipated, and 25 mg. of phenobarbital was injected slowly intravenously. No further seizures occurred. The patient's condition progressively improved, and the next morning the child showed no

neurologic abnormality but had an elevation of temperature and signs of a mild upper respiratory infection. History revealed that the child had previously had convulsive seizures with high fever on 2 occasions.

Comment. This child was not observed during the night following injury but showed signs of neurologic disturbance in the morning immediately before onset of a recognized convulsive seizure. The past history of convulsion with high fever suggests a lowered seizure threshold, and, although the temperature was not elevated on admission, the prodromal phase of his respiratory infection may also have been an aggravating factor.

Case 5. "Postcontusion seizure syndrome." R.S., a 4-year-old girl, was admitted to Saadi Hospital on November 2, 1960, after having fallen 6 ft., landing on her head, one-half hour previously. She was not unconscious but was restless, and a local physician recommended hospitalization. Within a few minutes after admission, the child had a convulsive seizure, following which she remained unconscious with left hemiplegia. The eyes were deviated to the left. Roentgenograms showed an extensive fracture extending from the vault into the right middle fossa and sphenoid wing. Swelling of the right orbital contents appeared rapidly, and there was fluctuant swelling beneath the scalp in the right frontal area. Surgery was alerted, blood was taken for crossmatching, and intravenous fluid was started. The child did not have further seizures, and her neurologic state began to improve slowly. Over the next four hours, the patient became more alert and the hemiplegia gradually receded. The following morning, the patient was neurologically normal except for the right orbital ecchymosis and swelling of the scalp. She had no further difficulty.

Comment. This child was admitted to the hospital in coma only half an hour after a severe cranial injury from which she did not lose consciousness. In view of the extensive fracture, it was possible that the patient had [1] a rapidly expanding extradural hematoma, [2] a cerebral contusion producing seizures with a superimposed developing hematoma, or [3] a cerebral contusion without other complication. During preparation for operation, it became evident that the patient was very gradually but progressively improving. This improvement was continuous, and the patient's clinical recovery was complete.

DISCUSSION

All of these children suffered head trauma of considerable magnitude, since 4 fell landing on the head and 1 was struck by a swinging door; 3 sustained skull fracture. However, in all cases, clinical evidence of primary brain injury was slight. Only 1 (Case 1) was briefly unconscious at the time of injury, and all were apparently neurologically normal after the injury for intervals varying from fifteen minutes (Case 1) to twenty hours (Case 4). Thus, neither the severity of the injury to the cranium nor the severity of injury to the brain can be relied upon in differentiation of the 2 conditions.

It is of interest that each of the children with seizures suffered direct trauma to the frontal portion of the cranium, and in each case focal neurologic signs were present contralateral to the superficial injury. This suggests that the brain lesion precipitating the seizure was probably subjacent to the area of direct trauma. Since this picture has been seen only in young children at an age when the cranium is rather malleable, it is postulated that deformity of the cranium at the moment of impact produces direct contusion of the cortex. Whether seizure follows such a contusion, and the interval of delay before its appearance, will be determined by the location and severity of the contusion, particularly in relation to the "motor" cortex. Factors relating to underlying seizure susceptibility may also play a role (Case 4).

When the child is seen in the critical postconvulsive state, differentiation from a surgical lesion may initially be impossible and preparations for surgical treatment must be started. However, if the possibility of postcontusion seizure is kept in mind, certain characteristics of the clinical history and course will lead to the correct diagnosis.

The most helpful differential information is historic, particularly if it can be established that the patient was neurologically normal immediately before onset of seizure or coma.

If the interval from injury to the onset of

coma is very short, as in Cases 1 and 5, it is not likely that an extradural hematoma would reach sufficient size to precipitate seizure within that period of time. However, in such cases, it is possible that, although the seizure did result from cerebral contusion, an extradural hematoma is simultaneously developing. Careful evaluation of the patient's clinical course will lead to the correct diagnosis. If the interval between injury and seizure is long, as in Case 4, it may be possible to establish that the child was neurologically normal up to the moment of onset of the seizure. This would also make extradural hematoma unlikely.

In many instances, the time intervals are intermediate and the historic information is unclear, so that differentiation initially is difficult. In such cases, the diagnosis will depend on careful and continuous observation of the patient. With progressive recession of the localizing signs and steady improvement in the level of responsiveness, it becomes increasingly probable that the underlying pathology is cortical contusion rather than massive hematoma.

Whether the seizure is secondary to a surgical mass lesion or to cortical contusion, it must be interrupted promptly and further seizures prevented. In controlling the seizure, overmedication is dangerous since it produces added depression and obscures the changing neurologic state of the patient. Thus, all anticonvulsant medication should be given intravenously. By this route, dosage can be titrated directly against the seizure, and the dangers of over- or undermedication will be avoided.

To date, none of the children in this series or of those seen previously with this syndrome[1] has had persisting difficulty with seizures after trauma. The low incidence of posttraumatic convulsive disorder attributable to closed head injury in all age groups supports the conclusion that this syndrome results from an acute focal disturbance which is self-limited if medically controlled and should not leave residual disability.

SUMMARY

Cases of head injury in five children are presented. In each case, the injury was followed by a "lucid" interval ending in coma. The clinical sequence was suggestive of extradural hematoma. All but one of the children proved to have minimal cerebral injury, presumably cortical contusion, from which they recovered under medical management. The fifth child had a massive extradural hematoma requiring surgical treatment.

The clinical differentiation and management of the two lesions are discussed.

CONCLUSIONS

A syndrome encountered in infants and children after head injury, characterized by delayed focal convulsive seizure, is described. This syndrome may mimic extradural hematoma.

In the "postcontusion seizure syndrome," the patient's critical condition is secondary to convulsive seizure rather than the result of cerebral compression.

If the syndrome is recognized and further seizures prevented, full recovery should occur.

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Case report

Fatal electrolyte imbalance secondary to cerebrospinal fluid fistula in an infant

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In 1932, Davidoff and Bancroft[1] reported a case of ureterodural anastomosis for the treatment of hydrocephalus. The child died nine days later. The shunt was patent, and the authors stated that death was apparently due to "dehydration and malnutrition."

Matson[2] appears to have been the first to clearly recognize that dehydration and electrolyte depletion could follow the loss of cerebrospinal fluid, especially in the infant. He and Dees,[3] concurrently reporting on the same group of cases, ascribed to this cause some of the deaths of infants in whom subarachnoid or ventriculoureteral shunts had been performed for hydrocephalus, and, since then, Matson[4,5] in both written and verbal discussion has repeatedly emphasized the importance of combating fluid and electrolyte loss in infants with such shunts.

The case reported here illustrates the importance of this factor and emphasizes the need for prompt and adequate treatment whenever there is persistent and profuse loss of cerebrospinal fluid, especially in an infant.

This 14-month-old white male infant was brought to the office by his parents. They stated that he had been born with an unepithelized lumbosacral myelomeningocele. Eventually, it epithelized but occasionally drained. It had slowly enlarged as had the head, but the child's general health had remained good.

Two weeks earlier, the child had fallen from a bed, with rupture of the epithelized sac of the myelomeningocele. Suture of the sac had been performed in another city, and the child had been given antibiotics.

Examination revealed a male infant whose head measured 52 cm. in diameter, while the chest circumference was 43 cm. The anterior fontanel was widely open and depressed. There was a large, collapsed, epithelized lumbosacral myelomeningocele, which was draining clear fluid. The child was irritable, cried lustily, and did not appear dehydrated.

He was admitted that same day to the Neurosurgical Service at Mercy Hospital, and blood chemistry studies were ordered. The following afternoon, the rectal temperature rose suddenly to 102.4° F., and the child became pale, with rapid pulse and labored respirations. In spite of stimulants, intravenous fluids, oxygen, and a blood transfusion, the child died approximately twenty hours after admission to the hospital.

Laboratory examination of the blood revealed 6,190,000 erythrocytes, 14,700 leukocytes, hematocrit of 43%, and hemoglobin of 13.9 gm. The blood potassium was 9.3 mEq. per liter; sodium, 118.7 mEq. per liter; total protein, 9.35 mg. per 100 cc.; albumin, 6.25; globulin, 3.1; and urea nitrogen, 15 mg. per 100 cc.

Necropsy revealed pulmonary atelectasis, edema, beginning bronchopneumonia, and the following congenital anomalies: lumbar spina bifida with myelomeningocele; Arnold-Chiari malformation with marked internal hydrocephalus; syringomyelia; bilateral talipes; congenital deformity of pelvis; adrenal hyperplasia; and undescended testes.

The primary cause of death was considered

From the Neurosurgical Service, Mercy Hospital, Chicago.

to be severe dehydration and electrolyte imbalance with acute pulmonary complications. The apparently satisfactory condition of the child up to the time of his collapse lulled his physicians into a sense of false security, so that active measures to combat the fluid and electrolyte loss were not instituted until too late.

The seriousness of continued loss of cerebrospinal fluid in an infant cannot be too strongly emphasized. It is possible that such loss may be serious in some older children and adults, especially if other factors such as coma, inadequate fluid or electrolyte intake, and vomiting or diarrhea exist. It is suggested that, in all cases of prolonged or persistent cerebrospinal fluid fistula, attention should be paid to possible dehydration and electrolyte imbalance and the necessary steps should be taken to prevent or overcome these complications. Matson[5] has recommended that, in an acute crisis, normal saline and one-half molar sodium lactate solution should be given rapidly intravenously. He further recommends that 2 gm. (one-half teaspoon) of table salt should be given daily as a prophylactic measure in cases of arachnoidureterostomy. This advice should be followed in any type of cerebrospinal fluid fistula in an infant or young child.

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4. Matson, D. D.: Ventriculo-ureterostomy. J. Neurosurg. 8:398, 1951.
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Neurology

BOOK REVIEWS

Neural Mechanisms of the Auditory and Vestibular Systems

edited by Grant L. Rasmussen and William F. Windle, 422 pp., ill., Charles C Thomas, Springfield, Ill., 1961. $15.75.

This monograph presents a series of 25 contributions to a well-planned symposium sponsored by the National Institute of Neurologic Diseases and Blindness. It is oriented toward elucidating the basic anatomy and physiology of hearing and equilibrium and achieves that goal by presenting authoritative discussions in every aspect of the field. It is essentially a manual for investigators, but the alert neurologist or neurosurgeon who wishes to be brought up-to-date on fundamentals and who seeks fertile ideas for clinical exploitation will read it carefully.

The scope of the book is well indicated by the title. There are chapters on the structure of the auditory and vestibular systems beginning with new data revealed by electron microscopy and concluding with the cerebellar and cerebral components, detailed discussion of new physiologic studies on these systems, including the role of afferent fibers to the receptor organs, and, finally, data on the histochemistry.

The contributors, who in effect summarize their experimental work and present it in broader scope than in the isolated papers, are editors Grant L. Rasmussen and William F. Windle and associates von Bekesy, Bordley, Brodal, Carpenter, H. Davis, de Lorenzo, Desmedt, Dohlman, Engstrom, Fernández, Gacek, Galambos, Gernandt, Gross, Guild, Hind, Lindsay, Livingston, Lorente de Nó, Neff, Palay, Rose, Schuknecht, Tasaki, Tonndorf, Tunturi, Wersäll, D. Wolff, and C. Woolsey. The foreword was written by R. L. Masland. The index is well prepared, and, for the convenience of the studious reader, there is one list of references for all contributions.

A.R.V.

Clinical Neurology

by Sir Russell Brain, 399 pp., ill., Oxford University Press, New York City, 1961. $8.75.

A newcomer to the neurology textbook scene in 1961 is this 399-page volume by the distinguished English neurologist with the appropriate name of Brain. In the first line of his preface, Sir Russell states that this volume is not a "smaller version" of *Diseases of the Nervous System*, a text well known to all serious students of neurology. His avowed purpose has been to provide a text for doctors and students "who require to know only the essentials of neurology." The author has achieved his objective in a well-written, compact form.

He has divided his material into 5 main sections. Part 1, under the caption of Disorders of Function, deals with history taking—a detailed description of the examination of the functions of individual cranial nerves and of the trunk and extremities in orderly fashion. He describes the pathologic deviations from the normal with appropriate neurophysiologic interpretations. This part of the text is particularly well done and commendable for its clarity. Other features of Part 1 concern various technical examinations to facilitate diagnosis. Consciousness and unconsciousness are discussed with special reference to the reticular system. The various disorders of sleep and

consciousness are briefly discussed. Epilepsy and headaches conclude Part 1.

Part 2 covers disorders of different systems and anatomic regions. Each chapter begins with a simple description of the anatomy and physiology of the region or system, followed by clinical discussion of disorders and syndromes common to the area. Part 3 is devoted in similar manner to infections; Part 4 covers diffuse disorders of hereditary, metabolic, and degenerative origin. Part 5 brings in the psychologic aspects of neurology. There are anatomic charts and photographs in support of the text material and a glossary of terms. The PNA nomenclature has been used throughout the text.

True to the principles of good medical writing, especially adhered to by British writers, is the simple language used, uncluttered by medical jargon. The format is attractive.

It is a text admirably suited to the needs of the beginning student in clinical neurology. The student with a deeper interest in this field will seek texts of more detailed coverage or other source material. The practicing physician will find *Clinical Neurology* by Brain a convenient, quick reference book.

M.G.M.

Handbook of Physiology—Section 1, Volume 3, Neurophysiology

Section Editor, H. W. Magoun, Editor in Chief, John Field, 572 pp., ill., Williams & Wilkins, Baltimore, 1961. $20.

Brought out by the American Physiological Society, the *Handbook of Physiology*, of which this is Section 1, Volume 3, was "designed as a repository for the body of present physiological knowledge systematically organized and presented." The current volume is the last of 3 covering the subject matter of neurophysiology. It contains 22 chapters, each the work of an authority; 11 of these, including 1 on psychosomatics, represent the *Handbook's* contribution to understanding of the higher functions of the nervous system. The remainder deals with topics of cerebral circulation, intracranial and intraocular fluids, neural metabolism from several viewpoints, chemical architecture, the chemical environment, and disturbances of neural function in the presence of congenital disorders. An excellent integration of the 3-volume work by R. W. Gerard forms the concluding chapter. The format is superior, and the illustrations are superb. The work is a necessity for those who would have a well-rounded library in the neurologic field.

J.L.O.

Multiple Sclerosis: Prognosis and Treatment

by Leo Alexander, Austin W. Berkeley, and Alene M. Alexander, 188 pp., ill., Charles C Thomas, Springfield, Ill., 1961. $7.50.

Multiple Sclerosis by Alexander, Berkeley, and Alexander is a new member of the American Lecture Series published by Charles C Thomas. It is a mathematical approach to the study of the disease developed by Leo Alexander, who sought a method to objectively follow the fluctuating course of the disorder; 554 cases of multiple sclerosis have been followed since 1948. By means of his scoring system and frequent evaluation of each individual case, the authors have been able to group the patients according to symptom complexes and severity of the process and to determine the prognosis for each individual case.

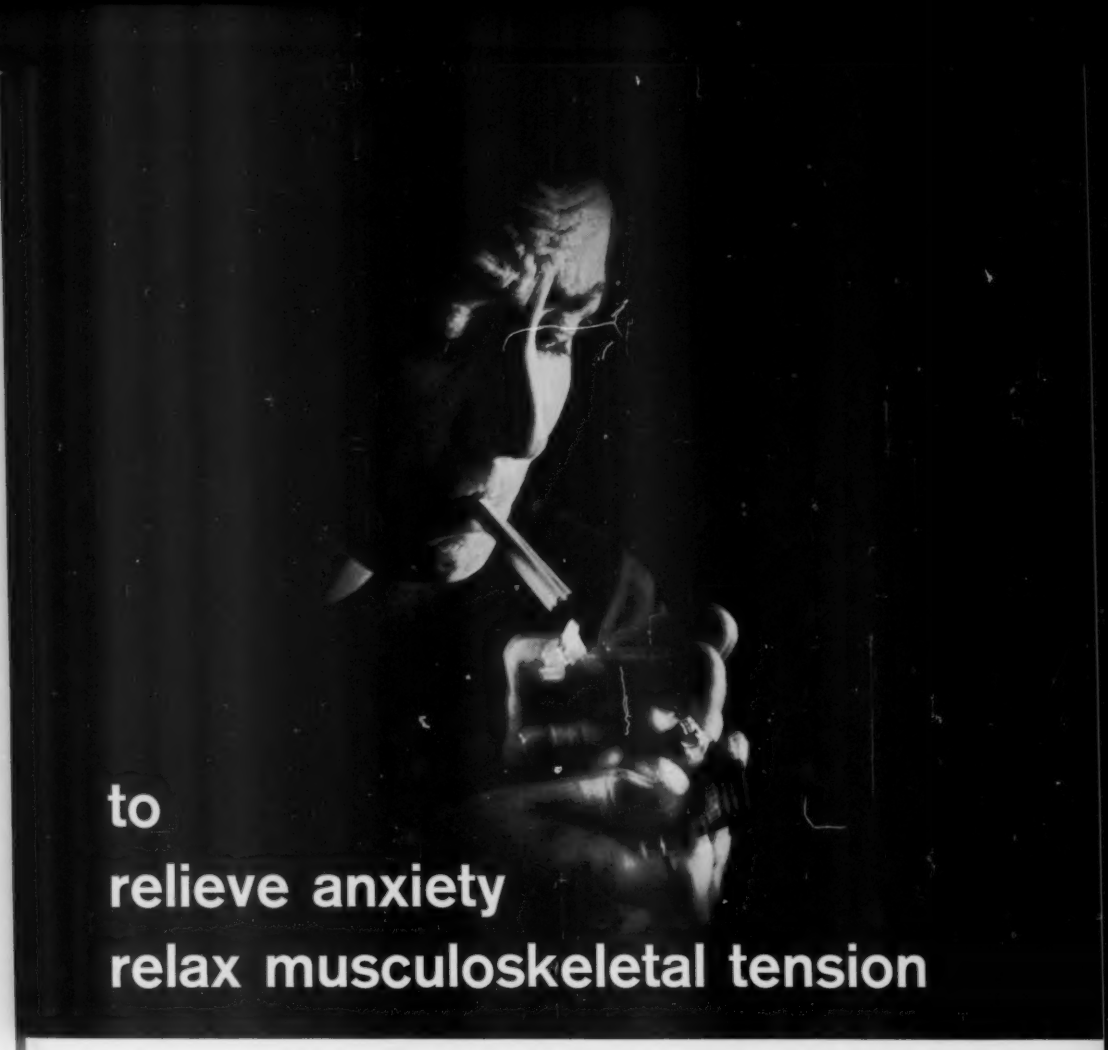
Numerical values have been assigned to each neurologic finding, with higher point values for findings of serious import and lower points for relatively less significant findings. For the completely disabled, the cumulative score would range from 500 to 700. The case in remission without objective findings would be scored at zero. In using this point system, 4 separate examiners were able to achieve high correlation values in their examination of patients, giving validity to the method.

By study of the individual attacks and the course of the disease, using the point system, classification and prognosis were determined.

The use of steroid therapy has been carefully evaluated, with the finding of ACTH having definite value for selected cases and blood transfusions giving improvement or remission in certain cases. Indications for both forms of treatment are given, and the methods are outlined. A chapter is devoted to the general management of the multiple sclerosis patient.

This interesting study should appeal to any physician dealing with this disease who desires a method of evaluation and planned therapy.

M.G.M.



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*Ayd, Frank J., Jr.: Drug-induced Extrapyramidal Reactions: Their Clinical Manifestations and Treatment with Akineton. Psychosomatics 1:143 (May-June) 1960.

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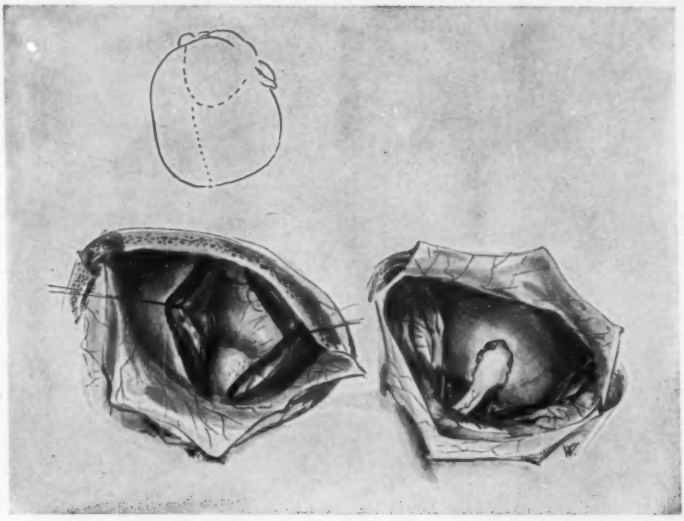

FIG. 148. *Glioma of right optic nerve in a 3-year-old girl before (right illustration) and after (left illustration) deroofing of orbit (approach via right transfrontal osteoplastic craniotomy). (Drawing)*

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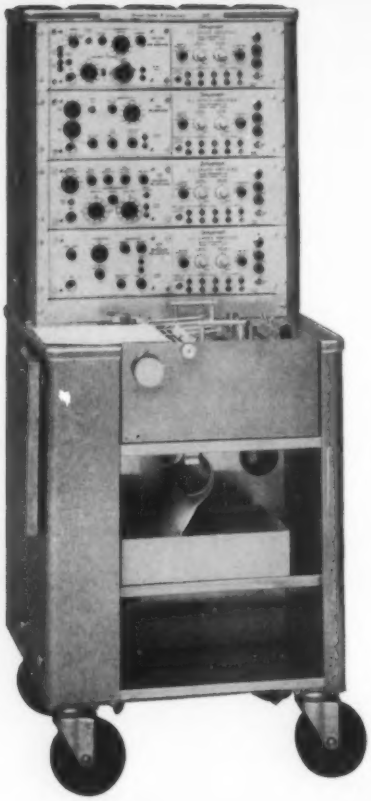

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THERAPEUTIC INDEX

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* K. R. Magee and M. R. Westerberg, **Neurology, 9**:348, 1959.

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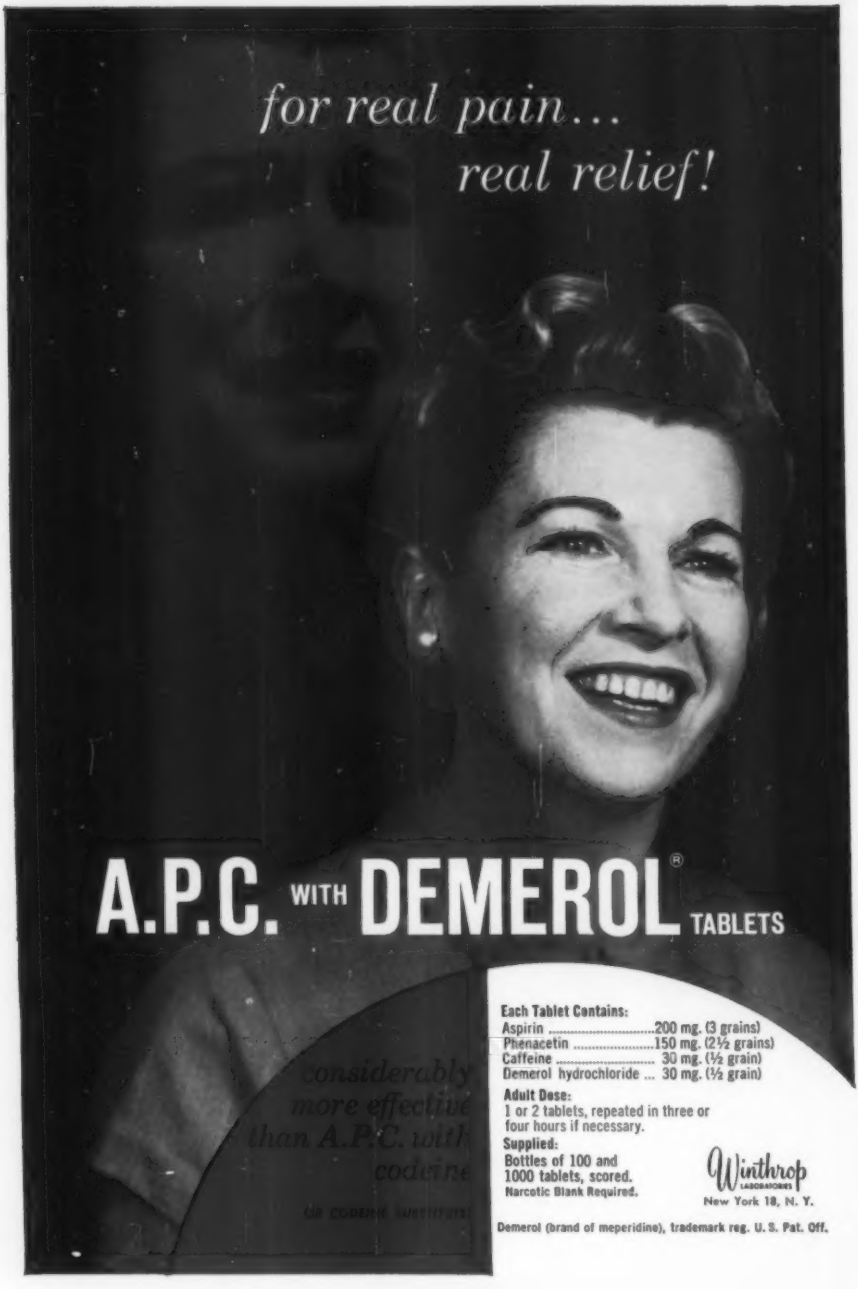
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